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Integrative Processes in Single Spinal Interneurones with Proprioceptive Connections

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INTRODUCTION

The activity of the spinal interneurones has been the subject of a great number of investigations by indirect methods such as analyses of reflex responses and slow cord potentials. A direct recording of the activity in single spinal interneurones with extracellular microelectrodes was first carried out by LLOYD (1941, 1942) who analysed interneurone systems connected with the pyramidal tract and long descending pathways, and further by RENSHAW (1946) who studied interneurones activated by antidromic ventral root stimulation.

The intracellular microelectrode technique (LING and GERARD 1949), successfully applied on motoneurones by BROCK, COOMBS and ECCLES (1952), gave rise to a new approach also to the analysis of interneurone functions. Employing this technique in investigations on cat, WOODBURY and PATTON (1952) studied the possibilities to distinguish between primary afferent neurones, interneurones and motoneurones by means of differences in their electrical properties and in their types of responses to electrical stimulation of dorsal roots. Further data on the electrical properties of the intracellularly recorded action potentials from spinal interneurones have been obtained in investigations by ECCLES, FATT and LANDGREN (1954, 1956), KOLMODIN and SKOGLUND (1954 a, b), FRANK and FUORTES (1955).

The experiments of KOLMODIN and SKOGLUND have primarily had in view a study of the functional organization of spinal interneurones by means of *natural* stimulation. The principal results of various phases of these investigations have been published in preliminary reports, one of which (1954 b) contains results from an analysis of two hundred interneurones in the ventral horn. Adequate stimulation of extero- and proprioceptors was shown to set up sustained interneurone discharges of up to 200 impulses per sec or to inhibit a pre-existent activity, whether spontaneous or induced from any known source. On the basis of the various convergence patterns a functional classification of the interneurones could be made and examples were given of typical convergence patterns in neurones with ipsilateral and/or con-

tralateral afferent connections. Descending activity from supra-segmental levels was also observed to converge on some of these neurones. By means of histological localization methods the position of the various neurones could be determined, and it was found, for instance, that neurones of reciprocal behaviour were often situated adjacent to each other.

In connection with the analysis of the central pathway for direct inhibition, ECCLES, FATT and LANDGREN (1954, 1956) have also studied certain convergence problems in single interneurones in the intermediate nucleus which could be influenced from group I and group III muscle nerves and from skin afferents. In a more extensive investigation, employing the intracellular technique on the cat's spinal cord, FRANK and FUORTES (1955) have studied the criteria of identification of various types of spinal neurones. In another work the same authors (1956) have dealt with functional aspects of the unitary activity of spinal interneurones. Their results are in accord with those mentioned above by KOLMODIN and SKOGLUND, as regards the type of response to electrical stimulation, the maximal frequency in sustained discharges during sensory stimulation and the existence of reciprocal innervation. Their investigation also included studies of interneurones activated by electrical stimulation of the ventral roots.

A second report of studies on spinal interneurones with chiefly exteroceptive connections has been published by SKOGLUND and KOLMODIN (1956), in which the principles of the convergence from exteroceptive sources of different modality and locus have been laid down.

In this connection it should be mentioned that a marked convergence, both excitatory and inhibitory, on single units in Flechsig's tract has been demonstrated both by means of electrical stimulation of muscle nerves (LAPORTE, LUNDBERG and OSCARSSON 1956) and by natural stimulation of proprio- and exteroceptors (LAPORTE and LUNDBERG 1956).

The present paper describes an investigation, by means of intracellular recording technique, of interneurones in the lumbar region of the cat's spinal cord which could be influenced by natural stimulation of muscular stretch receptors. The principal results have been published in a preliminary report (KOLMODIN 1956).

The investigation has been undertaken to study three main aspects of the function of the neurones under observation:

I. The various types of frequency responses elicitable in single interneurones by means of passive stretch of different muscles have been studied. Attempts have been made to find a method by which the impulse frequencies in the afferent fibres and in the interneurones could be compared. For this purpose muscle stretch within normal limits has been chosen as a simple physiological standard stimulus ("standardized stretch"). On the basis of recordings from a number of muscle afferents and interneurones activated in this way a comparison has been made of the average pre- and postsynaptic frequency-time curves; the frequency transformation represented by the curves is described as occurring in what is termed "an average interneurone"

II. In a number of interneurones, the functional organization of the convergence system of interneurones has been analysed by a systematic mapping of the various sources in the ipsilateral hindleg from which a given neurone could be influenced.

III. For a more detailed study of integrative functions, interneurones of a certain convergence pattern were examined by observing (*i*) the characteristic features of the frequency response of a single neurone to separate stimulation of different afferent sources, and (*ii*) the interaction of simultaneously induced activity from two different pathways converging onto a neurone. Integrative functions, such as summation and inhibition, previously studied by the methods of classical reflex physiology, could in this way be analysed quantitatively in single interneurones in terms of frequency modulation.

METHODS

Preparation. The experiments have been carried out on cats after decapitation under ether anaesthesia through transection of the spinal cord at the level of the atlanto-occipital membrane after ligation of the carotid arteries. The animals were curarized — except in some control experiments — and kept on artificial respiration. The spinal cord was exposed in the lumbar region and covered with paraffin or Ringer's solution. In most of the experiments the ipsilateral ventral roots from L_4 to S_1 were cut and, as they were being studied, placed on electrodes for antidromic stimulation. In a few experiments the skin and muscle nerves of the left hindlimb were dissected free in the usual way, kept under paraffin and placed on electrodes for artificial stimulation. In most cases natural stimulation was used, care being taken to limit the operative procedure as far as possible.

The animal was mounted in a special frame permitting rigid fixation which was obtained by means of pins in the crest of the ilium as well as in four of the lumbar vertebrae and by a clamp on one of the thoracic spinous processes. In a few experiments bilateral pneumothorax was performed in order to minimize the cord movements caused by respiration. However, if the trunk of the animal was hanging free from the bottom of the stand, and if the amplitude of the respiration movements was moderate, this operation was found to be unnecessary; in fact, it was sometimes observed to cause shock and was therefore abandoned.

The rectal temperature and the temperature in the pool of the spinal cord were continuously controlled, and the body temperature of the animals was kept constant at $38.5 \pm 1°$ C by means of heating lamps in suitable positions.

In one series of experiments the blood pressure was controlled. With appropriate amounts of liquid (Dextran and Ringer's solution) the blood pressure could usually be kept constant at about 90—100 mm Hg, even though the experiments lasted for 8 to 15 hours. By direct microscopical observation of the vessels in the pia arachnoidea it could be observed that the blood flow was slowed down and that "sludging" and "plasma skimming" occurred

when the blood pressure fell to about 60 (80—40) mm Hg. Simultaneously with lowered blood pressure there was always a lowered interneurone activity in the cord. In most experiments, the blood pressure was not recorded but, instead, the spinal blood flow was observed and as soon as it showed signs of being disturbed the experiment was discontinued.

In some experiments blood samples were drawn during the course of the experiment in order to determine the alkali reserve. Judging from these tests, the experimental procedure did not cause any significant changes in the acid-base balance of the blood.

Recording devices. The recording has been made via capillary microelectrodes filled with 2.7—3 M KCl solution. The capillary electrodes were drawn in a pulling device similar to that described by ALEXANDER and NASTUK (1953). The production of the electrodes and their properties are described in detail elsewhere (HAAPANEN, KOLMODIN and SKOGLUND 1957). In this connection it should only be mentioned that for the intracellular recording of interneurone activity electrodes are required with tip diameters well below 0.5 μ, and that the shape of the electrode tips is doubtless of no less importance than the dimensions of the electrode. The resistance of the microelectrodes was checked, after filling of the electrodes, by measurement with a vacuum tube testmeter and those having a resistance between 15 and 50 megohms were selected for use.

Fig. 1 shows the recording equipment in block diagram. The microelectrode was connected to an input stage (HAAPANEN and OTTOSON 1954) of cathode follower type which enabled an approximately ten-fold improvement of the high frequency response. At the beginning of each experiment the frequency of a sinusoidal test signal, at which the phase angle became 45 degrees (— 3 db), was determined. From this value and the known input capacitance of the input stage, the electrode resistance could be calculated. By the aid of square waves the frequency compensating controls were adjusted in order to get the best high frequency response from the recording system.

The main amplifier consisted of a direct coupled amplifier (HAAPANEN 1953) feeding a number of instruments namely: a double beam oscilloscope, an integrating counting rate meter with inkwriter (Siemens, type SD 12 K), another inkwriter of the same type recording the resting potential, a tape recorder (Grun-

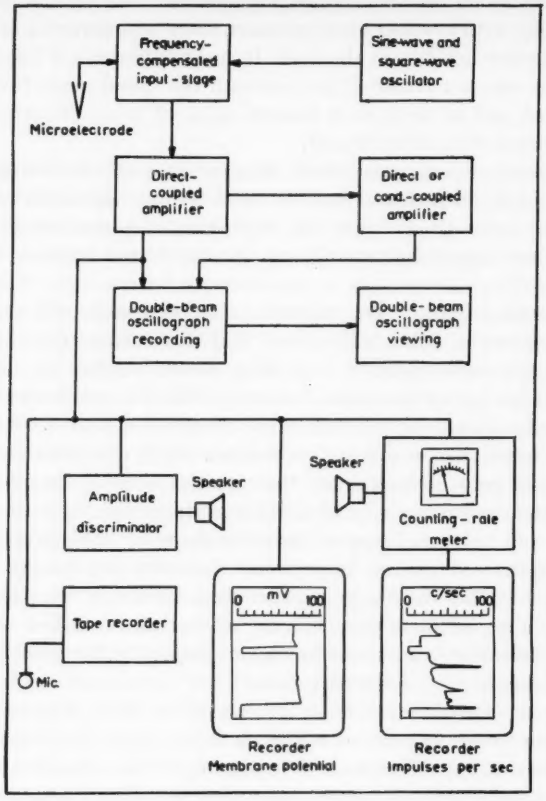

Fig. 1. Block diagram to illustrate arrangement of recording apparatus.

dig, type Tk 9) and a loud-speaker with adjustable pick-up level. To the output of the main amplifier was further connected a similar amplifier, being AC coupled and feeding the other beam of the oscilloscope. This arrangement made it possible to study the action potentials at higher amplifications when desired.

The rate meter is of the same type as is used in radiation measurements (*cf.* ELMORE and SANDS 1949), the instrument being, however, designed with the special application in mind. An amplitude discriminator is incorporated and the instrument can

thus be adjusted to respond only to impulses exceeding a preset amplitude. The rate meter is arranged to be paralyzed for 0.9 msec after the arrival of an impulse. The ranges chosen have been found convenient, the lowest one being 0—50 and the highest 0—1,000 pulses per sec.

Generally the time constant of the integrating circuit in the rate meter was kept sufficiently low (0.2 sec) to make the response time of 1/sec for the inkwriter the limiting factor.

The microelectrodes were mounted in a special holder on a Zeiss micromanipulator fixed at the stand in which the animal was mounted. The electrode was inserted into the spinal cord through a hole of about 1 mm^2 in the pia arachnoidea and could be slowly pushed downwards through the cord by means of the micromanipulator. The advancement of the electrode tip was read off on a micrometer gauge accurate within $\pm$ 1.5 μ. All manipulations were made under control of a binocular dissection microscope.

As a rule three small electrodes, at the most, were inserted into the cord of each animal between L_5 and S_1, one into each segment. After finishing the recording with a given electrode, it was left in its position in the cord. At the end of the experiment the animal was killed and the cord was fixed *in situ* by intravenous injection of 10 % formalin solution. The cord was then covered with the same solution and the preparation was left untouched for 12—24 hours. After that period the cord segments were dissected free and after further fixation *in vitro* serial frozen sections were made. In these sections the electrode track could be seen and the position of the various recording points could be determined by means of the values obtained from the micrometer gauge in the course of the experiment. In view of various obvious sources of error the exactness of this determination is not claimed to be greater than $\pm$ 100 μ.

Methods for application of natural stimulation. Natural stimulation has been employed in all but one of the various types of experiments described in this paper.

Stretch receptors in the muscle have been activated by passive extension of a muscle or of a group of synergistic muscles (*e. g.* soleus and gastrocnemius). In some cases the muscle has been stretched manually, by pulling at the prepared tendon; in other cases stretch has been applied by loads attached to the tendon. In preparations with intact tendon attachment, the

muscle was stretched by means of movements at the corresponding joints. Usually the tendons of the quadriceps and the triceps were cut and the tendons of the flexors of the knee and ankle joints were dissected free but kept intact. Whichever method was used, tension had to be applied with great caution. A rapid stretch usually induced small movements of the preparation, resulting in a "withdrawal" of the electrode from the neurone penetrated. The maximal tension in the muscle was usually obtained within $^1/_4$ to $^1/_2$ sec. A stretch of a muscle corresponding to maximal flexion or extension in the corresponding joint was chosen as standard stimulus ("standardized stretch"); this test represents a physiological stimulus strength and is easy to apply and reproduce.

In all these manipulations the leg of the animal was fixed in a suitable way by drills through the condyles of the femur and the tibia. The fixation could easily be released so that the leg could be moved freely if necessary; in some cases this was essential for the differentiation between various muscles or muscle groups. In those cases where no differentiation between the muscles with synergistic action on a certain joint has been made the term "muscle group" has been used. The flexor group of the ankle (tibialis anterior, peroneus longus and peroneus tertius) has been termed the tibialis anterior group.

Natural stimulation was also employed to activate pressure receptors, pressure being applied with a blunt instrument; pain receptors were activated by pin pricks or by squeezing the skin with a pair of forceps. Hair receptors were activated by touch of one or a few hairs with a brush or similar instrument.

In order to determine the position and the modality of the various afferent sources influencing a single neurone, a "checking list" was used, including individual stretch of the extensor and flexor groups of the ankle and knee joints, hair stimulation by stroking the fur of the whole leg, and pressure and pain stimulation on various points with a space interval of about 1 cm. When postsynaptic responses had been obtained by means of these manipulations, the position and the modality of the various kinds of receptors were studied in detail.

Identification of neurone types. The various types of spinal elements whose activity can be recorded by means of intracellular electrodes can be identified by their different electrical properties and their characteristic response to different types of stimulation

(WOODBURY and PATTON 1952, KOLMODIN and SKOGLUND 1954 b, FRANK and FUORTES 1955).

In the present paper, the term "interneurone" designates all neurones that are not afferent neurones nor motoneurones. The criteria applied for the distinction between these three main neurone groups will be fully described elsewhere (HAAPANEN, KOLMODIN and SKOGLUND 1957). A neurone that showed convergence or — if only one afferent source was found — responded to a single afferent volley by a repetitive discharge was thus classified as an interneurone.

Some further remarks on the differentiation between motoneurones and interneurones may, however, be appropriate in this connection. The classification is chiefly based on the fact that ventral root stimulation of motoneurones elicits a characteristic antidromic response which is missing in interneurones (BROCK, COOMBS and ECCLES 1952, *cf.* also ECCLES, FATT and KOKETSU 1954), but this "negative" criterion for interneurones may not always be reliable. Thus, *e. g.*, even a strong electrical stimulation of the ventral root may fail to excite certain fibres due to shunting; this applies especially to gamma fibres in view of their comparatively high stimulus threshold. Also, the axons to some of the motoneurones in a certain segment may leave the spinal cord through ventral roots at levels above or below the roots stimulated. Still another factor to be taken into account is that the electrode may be located in an axon collateral or a dendrite which is not invaded by the antidromic impulse.

It should also be kept in mind that at least four and usually five ipsilateral ventral roots have been cut in the experiments, which may considerably disturb the blood supply of the spinal cord, thus reducing the spinal excitability and the possibilities to obtain both anti- and orthodromic responses.

In the present investigation it has been regarded as a conclusive proof of a recording being made from single units when action potentials of constant shape and size have been recorded in combination with a stable membrane potential. The electrical characteristics of the intracellularly recorded action potentials have been fully described elsewhere (HAAPANEN, KOLMODIN and SKOGLUND 1957). In that connection it has also been discussed from what part of the cell — soma or axon — the recording is being made (*cf.* also FRANK and FUORTES 1956), a question which is of minor importance for the present analysis, since all conclu-

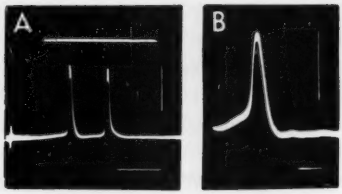

Fig. 2. Typical intracellular action potentials from two different spinal interneurones. *A*, repetitive response to electrical stimulation of gastrocnemius nerve; *B*, natural stimulation. *Vertical bar* 30 mV. *Time bar* in *A* 5 msec, in *B* 1 msec.

sions are equally relevant whether the recording is made from the cellbody or from the axon. The amplitude of the membrane potentials recorded has varied between 20 and 80 mV, the most frequent value being 40—50 mV. The values of the action potentials were as a rule 10 to 20 % lower than those of the membrane potentials. Some action potentials of the most frequent size and shape are shown in Fig 2.

Using intracellular recording technique, it has proved to be essential for a successful recording of stable membrane potentials that the size of the microelectrode is matched to the neurone size. The group of neurones recorded from in the present investigation is thus most likely a sampling of neurones based on size; it may reasonably be assumed that the smallest neurones from which successful recordings have been made are not below 20 μ in cell diameter.

The statistical analysis has been carried out with ordinary formulae (FISHER 1948).

RESULTS

I

Interneurone Discharges in Response to Muscle Stretch and Their Relation to the Afferent Inflow

As mentioned in the introduction, this first part of the investigation deals with the various types of interneurone discharges elicited by passive stretch of a muscle or a group of synergistic muscles and their relation to the afferent inflow.

1. THE AFFERENT INFLOW

In order to illustrate the background of these investigations a short survey will be given below of the receptors influenced by passive stretch of a muscle, as well as a brief discussion of the main differences in the afferent impulse patterns elicited during natural stimulation of receptors and during electrical stimulation of afferent nerves.

Survey of receptors activated during stretch of a muscle

Mammalian limb muscles (cat) contain two major stretch-sensitive receptor structures, muscle spindles and tendon organs (A and B endings of MATTHEWS 1933; tonic afferent fibres, KOBAYASHI, OSHIMA and TASAKI 1952). External stretch of a muscle evokes a discharge in spindle afferent fibres, the frequency of which is a function of rate as well as magnitude of stretch. In the tendon organs similar discharges are set up, the frequency of which provides a fairly direct measure of the tension in the tendon, whether due to contraction of the muscle or to application of external stretch (MATTHEWS 1933). The main difference between muscle spindles and tendon organs lies in their well-known behaviour during muscle contraction and efferent small nerve stimulation (MATTHEWS 1933; LEKSELL 1945; KUFFLER, HUNT and QUILLIAM 1951; HUNT and KUFFLER 1951). When maintained stretch is applied to a de-efferented muscle, both types of recep-

tors respond with a sustained regular discharge of impulses whose initial frequency may be as high as 200—300 impulses per sec; during maintained constant stimulation the discharge is then reduced exponentially within certain limits. The load-frequency relationship as well as the threshold of steady tension vary in different types of receptors and also in receptors of equal type in one and the same muscle (MATTHEWS 1933). Impulses from these slowly adapting receptors are chiefly mediated via afferent fibres belonging to group I and group II, whose conduction speed varies between 120—72 and 72—12 m/sec. (LLOYD and CHANG 1948; MERTON 1953; HUNT 1954.)

In ligaments and capsules of the joints as well as in the neighbourhood of tendons there are also receptors responding to stretch or pressure by a slowly adapting discharge (*e. g.* ADRIAN and UMRATH 1929; KOBAYASHI, OSHIMA and TASAKI 1952; BOYD 1953; BOYD and ROBERTS 1953; ANDREW 1954; COHEN 1955/1956; SKOGLUND 1956). In view of the anatomical site of these receptors in or adjacent to the tendinous insertions of muscles they are also very likely to start discharging in response to external stretch of a muscle.

Maintained stretch of a muscle also elicits discharges from *rapidly* adapting stretch-sensitive endings in the musculature (*e. g.* type C endings, MATTHEWS 1933), near tendons, in capsules of the joints and ligaments, *e. g.* the Pacinian corpuscles (GRAY and MATTHEWS 1951; *cf.* also KOBAYASHI, OSHIMA and TASAKI 1952; BOYD and ROBERTS 1953; BOYD 1954; SKOGLUND 1956). Such discharges consist of a burst of impulses which, as a rule, ceases within 1 sec after application of stretch.

In view of the facts indicated above, the total afferent inflow from a muscle subjected to rapid constant stretch can be assumed to consist of a complex, asynchronous discharge of impulses, varying according to several factors, such as different types of active receptors, the number of active fibres from these receptors, as well as the actual discharge frequencies and conduction speeds of the fibres engaged. Generally, however, the inflow may be described as follows. During the initial stage, after the rapid extension of the muscle, there is a swift increase of the total number of afferent impulses in the nerve; then, as the muscle is constantly loaded, the number of impulses rapidly decreases, due to the fact that the discharge frequency in some fibres is reduced or falls to zero, according to the rate of adaptation of the recep-

tors. During this initial stage there is thus a continuous variation of the afferent impulse frequency which must be considered as physiological both in regard to types of fibres activated and frequency. During the second stage, when the discharge from rapidly adapting receptors has ceased and the slowly adapting receptors have reached a fairly steady rate of discharge, the afferent inflow is still asynchronous. In this stage the frequency of the single fibres is constant, as is also the number of active fibres; the distribution of active and inactive fibres is physiological.

During repetitive electrical stimulation of an afferent muscle nerve, on the other hand, an afferent inflow is induced, consisting of waves of impulses that are practically synchronous in all fibres influenced. The temporal distribution of the impulses reaching the cord is exclusively dependent on the different conduction speeds in the fibres activated. All fibres have the same impulse frequency, and the distribution of active and inactive fibres is most likely un-physiological, as, during electrical stimulation, the number and the type of active fibres depend on the various electrical thresholds of the fibres. There is an obvious difference between an artificial inflow of this type and what is known of the afferent inflow during natural stimulation.

Frequency-time curves of afferent discharges from slowly adapting stretch receptors in muscle

The application of the counting rate meter, allowing a direct recording of discharge frequency-time curves during the course of an experiment, has made it possible to obtain an immediate survey of different receptor responses and their variations in a large number of afferent fibres. It has also been possible by this method to verify most of the various types of receptor discharges set up by muscle stretch described in earlier investigations.

Responses have been studied from practically all muscles in the cat's hindlimb. The material presented in this paper includes recordings from 75 fibres in the dorsal root and dorsal column and has been selected from a study of certain muscles, *viz.* triceps, the tibialis anterior group and biceps-semitendinosus. These have been chosen because a corresponding number of interneurones were found that could be activated from receptors in these very muscles.

The material has also been limited to discharges from slowly adapting receptors. No systematic attempt has been made to

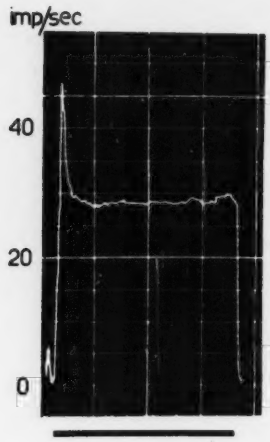

Fig. 3. Frequency-time curve obtained from single afferent fibre in dorsal root in response to standardized stretch of triceps surae. Recording by counting rate meter and inkwriter. *Solid line:* duration of stretch. *Time* between vertical lines 30 sec.

distinguish between different types of these receptors; no doubt both muscle spindles and tendon organs have been represented.

Only recordings with stable membrane potentials have been included in the material which indicates that it represents mainly recordings from coarse afferent fibres.

On the material thus chosen has been calculated an average frequency-time curve of afferent discharges from slowly adapting muscle receptors. This curve constitutes an attempt at a definition of the type of iterative afferent stimulation of the central neurones in the spinal cord produced by "standardized" muscle stretch.

A typical frequency-time curve for a discharge in an afferent fibre in response to stretch of a muscle (triceps) recorded with the aid of the counting rate meter and the inkwriter is illustrated in Fig. 3. Following the onset of stretch there is a rapid increase of the response frequency to a maximum of about 50/sec and then again a slow characteristic exponential decrease, until, after about 15 sec, a fairly constant level (*cf.*, however, p. 21) is reached which is maintained during the period of steady tension, in this case about 100 sec.

During repeated application of "standardized stretch" minor

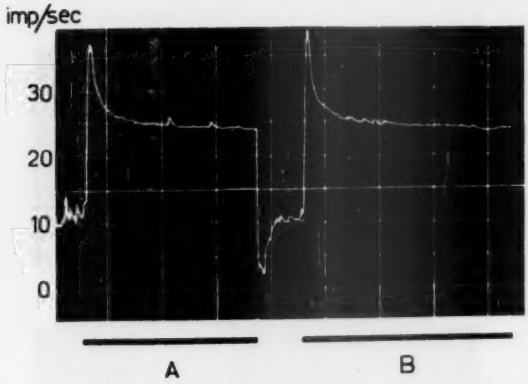

Fig. 4. Frequency-time curve obtained from single afferent fibre in dorsal root in response to repeated (*A* and *B*) standardized stretch of triceps surae (same experiment as in Fig. 3 but another fibre). Note slow return of resting discharge, of 10 impulses per sec, on release of muscle. *Solid line:* duration of stretch. *Time* 30 sec.

variations may appear in the response, *e. g.* in the initial maximal frequency, but the general shape of the curve is very uniform. This is illustrated in Fig. 4, showing recordings from another fibre from the same muscle.

Both Figs. 3 and 4 represent frequency-time curves of the most common type, characterized by a relatively rapid adaptation to a constant level of about 25—30/sec (*cf.* average curve for triceps, Fig. 8).

Fig. 5, on the other hand, demonstrates frequency-time curves from four fibres (still from the triceps muscle in the same experiment) which deviate considerably from the type curve. These curves give an idea of the variations sometimes seen in discharges in different fibres from one and the same muscle both in regard to absolute frequency values and in the rate of adaptation. They are also typical of the degree of variations in discharge types found in slowly adapting receptor responses from *different* muscles.

Special features. The results described so far are in accordance with earlier findings by MATTHEWS (1933). Before passing on to a description of the average frequency-time curve established on the basis of a number of recordings from fibres in different muscles, a brief survey will be given of some special features in regard to receptor discharges that are of importance for the analysis of postsynaptic effects.

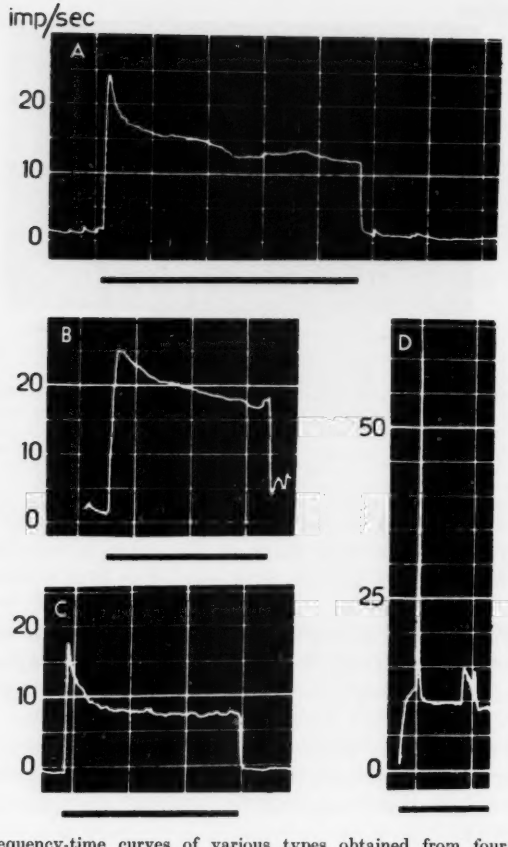

Fig. 5. Frequency-time curves of various types obtained from four different fibres (*A*—*D*) from triceps surae in response to standardized stretch (same experiment as in Figs. 3 and 4). *Solid line:* duration of stretch. *Time* 30 sec.

Matthews found that the muscle spindles (type A receptors) could be made to discharge by a light pressure of the belly of a muscle. A typical response of this kind is illustrated in Fig. 6, where the suddenly appearing irregular frequency fluctuations have been produced by means of a light pressure on the belly of the muscle with a blunt instrument. This observation has been used in the present work for a rough localization of the active receptors.

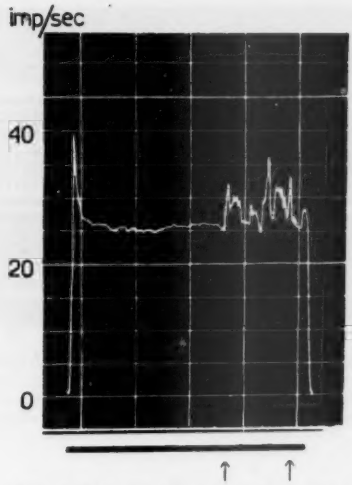

Fig. 6. Increase of adapted discharge frequency after repeated slight blunt pressure applied (between arrows) to belly of the stretched tibialis anterior muscle. *Solid line:* duration of stretch. *Time* 30 sec.

Another observation by Matthews is verified in the experiment illustrated in Fig. 4. The curve shown is from a fibre discharging at a rate of about 10 impulses per sec even when the muscle is relaxed. After the muscle has been loaded for about 90 sec (solid line *A*) and then rapidly unloaded, the response frequency immediately falls to the vicinity of zero and then rises slowly again to the original resting level. The course of this recovery is of interest for a comparison with postsynaptic frequency variations induced in a similar way (*cf.* Fig. 18).

Matthews also observed that a high constant loading of a muscle sometimes resulted in a slow increase of the response frequency after 1—2 min. According to Matthews this phenomenon was likely to be due to impeded circulation. A similar increase has sometimes been observed also in this investigation, as shown in Fig. 3. In this case the frequency increased slowly from 27 to 30 impulses per sec during the adapted level. It should be pointed out, however, that in these experiments the muscles have never been subjected to supramaximal stretch, the degree of stretch always being within the physiological limits indicated above.

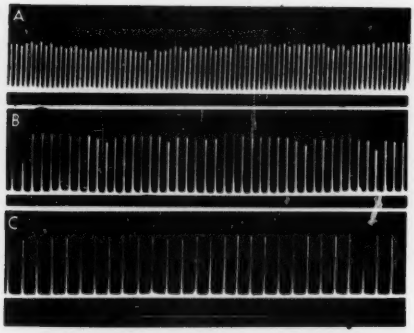

Fig. 7. Typical regular rhythm of adapted discharge of three different fibres in response to maintained stretch of triceps surae. *A* and *B*, standardized stretch; *C*, 90° flexion of ankle joint. Oscillographic play-back of intracellular action potentials recorded on tape. *Time bar* 0.5 sec. Baseline slightly retouched.

As appeared from all these figures, the frequency variations during the adapted level are very small. Recordings of the action potentials in the usual way, as illustrated in Fig. 7, indicate that these variations are below 10 % which is in accordance with previous observations. The fact that the variations always remain small can be taken as a sign that the loading of the muscle has been fairly constant. The results also confirm that the ventral roots have been completely cut off, thus eliminating irregular effects on the discharges from the muscle spindles caused by gamma fibre activity (ELDRED, GRANIT and MERTON 1953).

The average frequency-time curve. The average frequency-time curve for slowly adapting stretch receptors in the muscle has been determined (*i*) on the basis of 75 recordings selected from fibres from the extensor and flexor groups of the ankle joint (triceps and tibialis anterior) and the flexor groups of the knee joint (biceps-semitendinosus), and also (*ii*) separately on the basis of 18 fibres from triceps. The course of the curve has been followed for 60 sec from onset of stretch. In all recordings "standardized stretch" has been applied, *viz.* stretch corresponding to maximal flexion or extension in the joint in question. The average curve has been drawn from the mean frequency values of the discharge in individual fibres at varying periods after onset of the discharge, the first value being measured after 1.5 sec and those following with 3 sec interval.

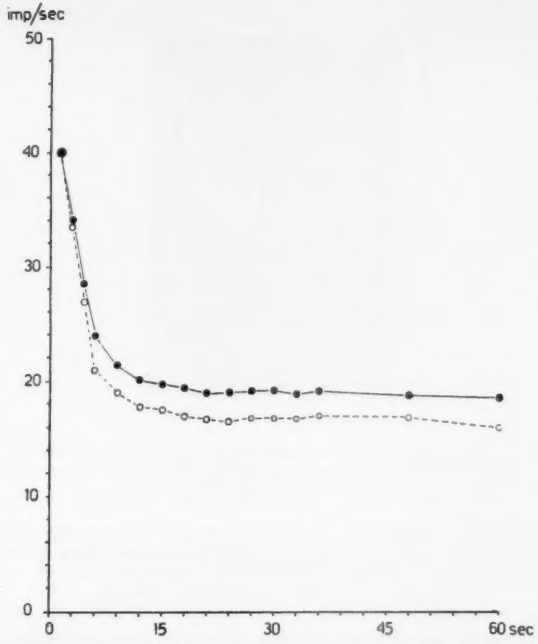

Fig. 8. Average frequency-time curves of afferent fibres in response to standardized stretch. *Filled circles:* mean values of discharge frequencies of 75 fibres from extensor and flexor groups of ankle joint and flexor group of knee joint. *Open circles:* mean values of discharge frequencies of 18 fibres from triceps surae. All measurements made on frequency-time curves directly recorded via the counting rate meter. *Abscissa:* time in sec after onset of discharge.

As appears from Fig. 8 the shapes of the two curves — the one for all muscle groups and the other one for triceps alone — on the whole correspond with each other. The initial frequency decrease is in both cases ended within the first 15 sec and after that period the frequency level is fairly constant. The turning point from the falling phase to the constant plateau of the curve — which is a rough measure of the rate of decline of adaptation in the receptors — is of great interest for later comparisons and has therefore been analysed statistically.

In view of the exponential decay of the receptor discharges it may often be difficult to determine the exact point where the falling phase is ended and the constant phase sets in. Therefore, the point where the impulse frequency of the single fibres reached

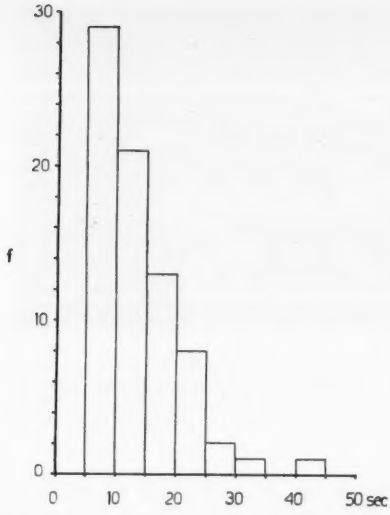

Fig. 9. Frequency distribution of "turning points" of frequency-time curves obtained from recordings of discharges from 75 afferent fibres in response to standardized stretch. *Ordinate:* frequency of occurrence; *abscissa:* time from onset of discharge in 5 sec class intervals.

110 % of their final constant plateau level has been chosen as the turning point. For the upper curve, from all muscle groups, the mean value of the turning point thus defined proved to be 13.5 ± 0.8 sec ($n = 75$, $s = 7$) after the start of the discharge.

Fig. 9 shows the frequency distribution of the periods thus measured in 5 sec class intervals. For 38 % of the fibres (29 fibres) the turning point was found to be within 10 sec and for 95 % of the fibres within 25 sec after the start of the discharge. Only in 4 cases did the value exceed 25 sec. The individual frequency curves for two of these cases are presented in Fig. 5 (*A* and *B*).

2. INTERNEURONE DISCHARGES

Ninety per cent of the interneurones activated by maintained passive stretch of a muscle showed sustained discharges; this group will be described below. Half of these neurones were initially silent, whereas the rest had varying rates of spontaneous activity. The interneurones responding by transient discharges, constituting ten per cent, are described on pp. 36—37.

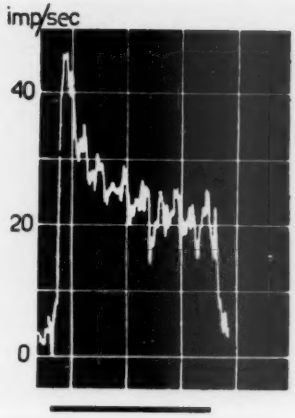

Fig. 10. Typical frequency-time curve obtained from dorsal horn interneurone activated by standardized stretch of triceps surae. *Solid line:* duration of stretch. *Time* between vertical lines 30 sec.

A. Sustained Discharges

Effect of application of steady stretch

The most common type of sustained discharge obtained in response to "standardized stretch" has a comparatively high initial frequency declining discontinuously to a lower level. An example of a postsynaptic response of this kind is given in the frequency-time curve, Fig. 10. The neurone is activated by standardized stretch of the triceps surae. As a result of the comparatively rapid stretch of the muscle, the discharge frequency rises rapidly to about 45/sec; during maintained stretch it declines, reaching a fairly constant level — although with typical fluctuations — after about 50 sec (see below). Usually the discharges have been studied for 60 sec but in some cases it has been possible to follow them for more than 6 min, only a moderate decline in frequency occurring during this period. No attempt has been made to investigate how long the discharge can be maintained at a relatively constant frequency. The frequency changes observed in these neurones in response to steady stretch are thus fundamentally similar to those recorded from slowly adapting muscular stretch receptors. There are, however, certain essential differences which will be discussed below.

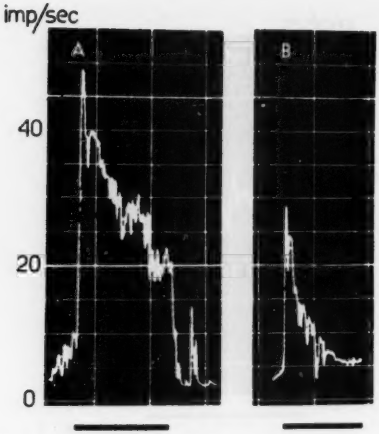

Fig. 11. Frequency-time curves obtained from two different interneurones activated by standardized stretch of: *A*, tibialis anterior (note stepwise decay of response frequency), and *B*, quadriceps. *Solid line:* duration of stretch. *Time* 30 sec.

During repeated standardized stretch, both the initial response frequency and the general shape of the curve are very constant in one and the same cell, as is the case also in the afferent fibres.

The frequency-time curves of the different neurones may, however, show considerable individual variations. In most cases the decline of the curve occurs as shown in Fig. 10. In many neurones, however, there is a marked stepwise fall of the response frequency like that illustrated in Fig. 11 *A* (*cf.* also Fig. 31). The types of decay demonstrated in the two figures may be observed in different interneurones in one and the same preparation; the fluctuations are thus not likely to be due to excitability changes in the spinal cord owing to the experimental procedure.

The response frequency in the "adapted" part of the curve has as a rule been about 20/sec or slightly higher, as shown in the figures. However, considerably lower values have also been observed, as is illustrated in Fig. 11 *B*, showing an adapted response frequency as low as 5—6 impulses per sec. Occasionally rates of discharge down to 1 per 1—2 sec have been observed. Such low frequencies have been more common in animals whose general condition had deteriorated, *e. g.* due to a fall of the blood pressure, but they have also occasionally occurred in animals in perfectly good condition.

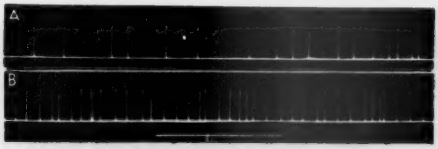

Fig. 12. Irregular spacing of impulses in discharge from single spinal interneurone. *A*, resting discharge; *B*, discharge 30 sec after application of standardized stretch to tibialis anterior muscle. *Time bar* 0.5 sec.

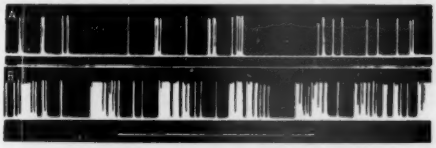

Fig. 13. Irregular spacing and grouping of impulses in discharge from single spina interneurone. *A*, resting discharge; *B*, response to stretch of biceps-semitendinosus muscle. Note distinct grouping of impulses in both *A* and *B*. *Time bar* 1 sec.

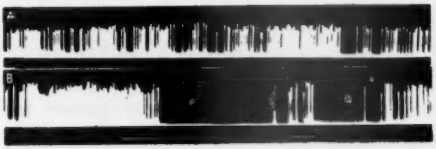

Fig. 14. High frequency discharges from two different interneurones activated by large stretch of: *A*, triceps surae; *B*, biceps-semitendinosus. *Time bar* 1 sec.

As appears from all frequency-time curves recorded direct via the counting rate meter, the postsynaptic response frequency is very irregular both during the falling phase and the plateau phase. As also appears from these curves, the amplitude of the frequency fluctuations also differs considerably in different neurones. The records shown in Figs. 12—14 have been selected to illustrate these irregularities in detail. Fig. 12 *A* shows the resting discharge in a spontaneously firing interneurone that could be activated by stretch of tibialis anterior. Fig. 12 *B* shows the discharge 30 sec after application of standardized stretch. As is seen, there is a moderate irregularity both in the pre-existent and in the induced activity. The records in Fig. 13 — from another neurone — illustrate a higher degree of irregularity with a tend-

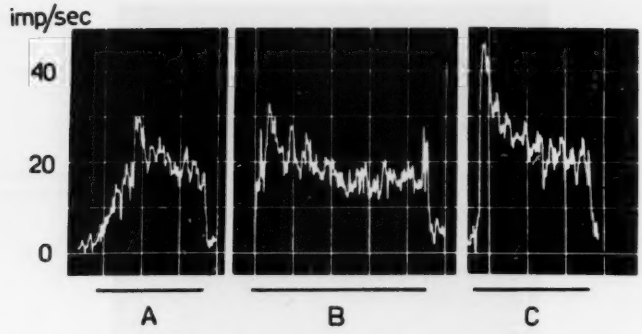

Fig. 15. Effect of variations in rate of stretch. Frequency-time curves obtained from interneurone activated by 6 mm stretch of triceps surae. Slow increase of stretch during: *A*, 20 sec, and *B*, 10 sec; *C*, stretch applied in less than $^1/_2$ sec (*i. e.* standardized stretch). Fig. 10 shows same interneurone. *Solid line:* duration of stretch. *Time* between vertical lines 30 sec.

ency towards periodicity. Even in the resting discharge (*A*) there is a certain grouping of the spikes which is still more pronounced when the neurone is activated (*B*).

Also at higher frequencies there is a marked irregularity in the discharges, as shown in Fig. 14; the records are from two different neurones activated maximally by means of loading exceeding the standardized stretch.

Effect of variations in rate and magnitude of stretch

The initial postsynaptic response frequency depends both on the rate of stretch and the magnitude of stretch applied to the muscle. The effect caused by variations in the *rate of stretch* is illustrated in Fig. 15. In record *A* the muscle has been subjected to a manual stretch which was very slowly increased for about 20 sec up to a tension corresponding to standardized stretch. In record *B*, the same rate of stretch was reached in about 10 sec and in *C* the same stretch was applied in less than $^1/_2$ sec.

As mentioned above, the standardized stretch used in this investigation was usually attained within $^1/_4$ to $^1/_2$ sec. If a more rapid stretch is applied, by hand or by means of an electromagnetic puller, the initial response frequencies obtained are higher, as illustrated in Fig. 16. The picture shows the response to stretch (*A*) applied at first very slowly for 10 sec (between arrows 1 and 2) and then more rapidly. At *B*, stretch has been

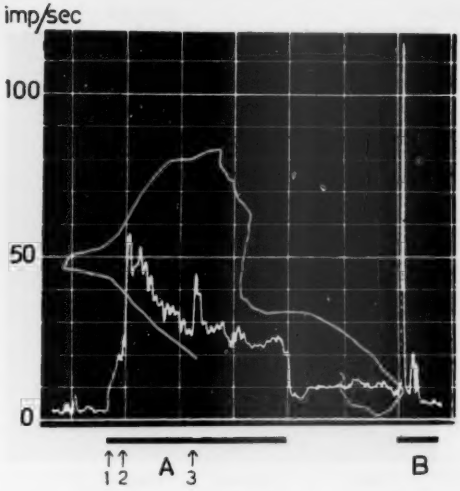

Fig. 16. Frequency-time curve obtained from interneurone activated by stretch of quadriceps. *A*, application of stretch slowly at arrow 1, more rapidly at arrow 2; at arrow 3 sudden increase of maintained stretch. *B*, maintained stretch applied by quick pull. *Solid line:* duration of stretch. *Time* 30 sec.

applied by a quick pull; in this case the initial response is twice as high. However, the rapid stretch caused movements in the cord resulting in a withdrawal of the electrode from the cell. The rest of the curve is, therefore, not recorded. As shown in these experiments, the more rapid the stretch the higher is the initial response frequency.

The effects of changes in the *magnitude of stretch* are demonstrated in Fig. 17, showing an interneurone activated by stretch of the triceps surae muscle. In this case approximately the same rate of stretch was applied at *A*, *B* and *C*. With an increased degree of stretch there is a rise in the response frequency. The discharge frequency can thus be modulated at will in these neurones by manually increasing or decreasing the degree as well as the rate of stretch applied.

If a constant tension of a muscle activating an interneurone is suddenly increased to the level of a higher, also constant tension, there is, as a rule, an increase of the postsynaptic response, followed by a comparatively rapid adaptation to the additional

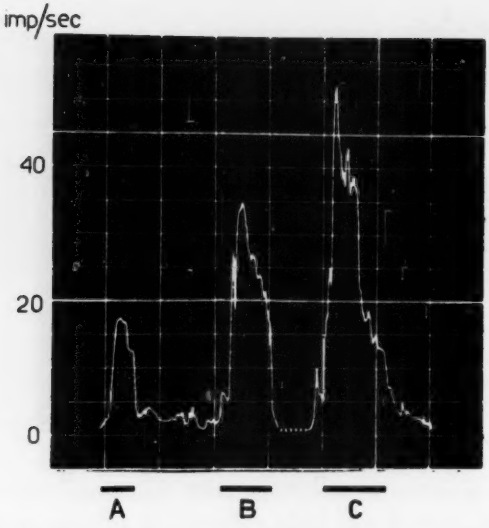

Fig. 17. Effect of variations in magnitude of stretch. Frequency-time curve obtained from interneurone activated by stretch of triceps surae. Magnitude of stretch in *A* 3 mm, in *B* 5 mm and in *C* 7 mm. *Solid line:* duration of stretch. *Time* 30 sec.

stretch. This is illustrated in Fig. 16, in which the load was increased (arrow 3) when the postsynaptic discharge had adapted to a certain tension.

In this connection it should be mentioned that in a few neurones the sustained discharge caused by stretch of a muscle suddenly ceased when the tension was further increased.

The *effect of rapid release of stretch* has been studied in a number of experiments, a typical example of which is shown in Fig. 18. The neurone was discharging spontaneously at a rate of about 20 impulses per sec. At arrow 1 standardized stretch was applied and at the second arrow the load was reduced to a lower tension, resulting in a fall of the response frequency to 15 per sec (dip of curve); then there is again a slow frequency rise up to a fairly constant level of 22 per sec. Both the fall after reduced tension and the following spontaneous rise in response frequency exhibit the same general time course as shown in the corresponding experiment when recording from a fibre (*cf.* Fig. 4).

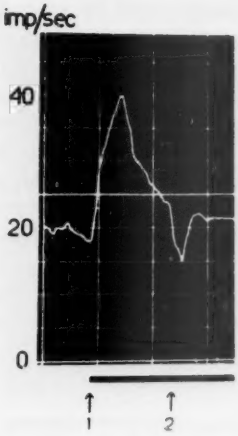

Fig. 18. Effect of rapid release of stretch. Interneurone discharge evoked by loading tendon of tibialis anterior with 300 g at arrow 1. Loading reduced to 200 g at arrow 2. *Time* 30 sec. Integrating time constant 0.8 sec.

Some observations on spontaneously discharging neurones

The description given above of the discharge pattern of the interneurones in response to passive muscular stretch is applicable both to initially silent cells and to cells with a resting discharge. Some examples will be given below of typical resting discharges, as well as some comments as to their origin.

The resting discharges were always irregular, as shown in Figs. 12—13. Their frequency was, as a rule, between 2—10 impulses per sec and seldom exceeded 15 per sec. In more than 50 % of the neurones the resting discharge could be abolished by a systematic relaxation of various muscle groups that might have been stretched when the joints were being fixed to the stand or in some other way.

In some of these spontaneously active neurones responding to muscular stretch by a typical discharge, a complete release of stretch was observed to result in a frequency fall to zero or to the vicinity of zero; the frequency then gradually increased again up to or slightly below its previous rate even in the absence of any obvious tension of the muscle. This is thought to be due to a similar mechanism as that described above in Fig. 18 (*cf.* also Fig. 4).

It was sometimes found that in an initially silent neurone a

slow spontaneous discharge could be evoked when the neurone had been activated by repeated stretch of the muscle.

As will be described in the second part (II), many of the interneurones studied could be activated by stretch of *more than one muscle group*. This was slightly more common in the spontaneously active neurones than in those silent.

In some cases it was not possible in this way to determine a probable peripheral source of the spontaneous activity. In most cases, however, the source could be traced to the resting activity of one of the muscles connected functionally to the neurone in question. These findings support the view that in many of these interneurones the spontaneous activity is due to a resting discharge in the slowly adapting receptors in the muscle of the kind described by MATTHEWS (1933).

Localization of receptors

No systematic attempt has been made in this work to distinguish between muscle spindles and tendon organs by means of, *e. g.*, stimulation of efferent fibres because this may, in fact, cause quick muscle movements that may be transmitted to the spinal cord.

As described in the previous section on the afferent fibre activity, the position of active endings was roughly localized by pressure on the muscle with a blunt instrument. When repeating this in experiments on interneurones, a region can be localized where pressure produces a short postsynaptic response or an acceleration of the response to steady stretch. Fig. 19 shows a frequency-time curve from an interneurone activated by standardized stretch of the triceps surae muscle. In the latter part of the response, when the frequency has reached a fairly constant level (arrow), the sudden rise in the postsynaptic response frequency has been obtained by light pressure on the belly of the muscle with a blunt instrument. This method allows only of a rough localization, it is true, but when testing in this way it was found that about 60 % of the slowly adapting postsynaptic responses studied could be elicited from the belly of the muscle; the rest seemed to have their origin in the neighbourhood of the tendons or in the joint capsules.

In some experiments attempts were made to dissect free the muscle fasciculus containing the receptors. This was done in the following way. At first the receptors were localized to a certain

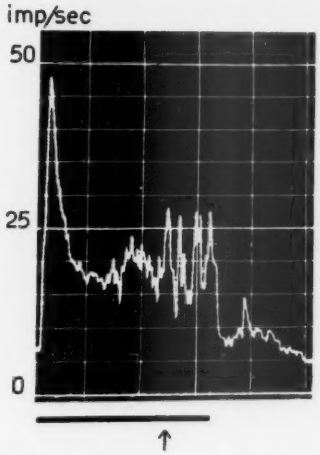

Fig. 19. Frequency-time curve obtained from interneurone activated by standardized stretch of triceps surae. From arrow onwards repeated light pressure applied to belly of muscle. *Solid line:* duration of stretch. *Time* 30 sec.

group of muscles, by means of passive stretch and blunt pressure. In an actual case the postsynaptic response proved to be of the type described in Fig. 10. By cautiously cutting one bundle of muscle after the other, a point was finally reached where the slightest touch increased the postsynaptic discharge. After cutting also this bundle there was no postsynaptic discharge in response to stretch in this muscle. In view of the position of these receptors in the middle of the muscle as well as their high sensitivity to pressure and stretch, they were certainly stretch receptors in the muscle and probably muscle spindles.

The average frequency-time curve

Also for the sustained postsynaptic discharges average frequency-time curves have been calculated, thus enabling a comparison with the corresponding curves for slowly adapting afferent discharges. The curves have been calculated on the basis of the sustained discharges from 67 different neurones activated by standardized stretch of the extensor and flexor groups of the ankle joint and the flexor group of the knee joint. The recordings have been selected in the same way as those used for the calculation of the average frequency-time curves for afferent fibres.

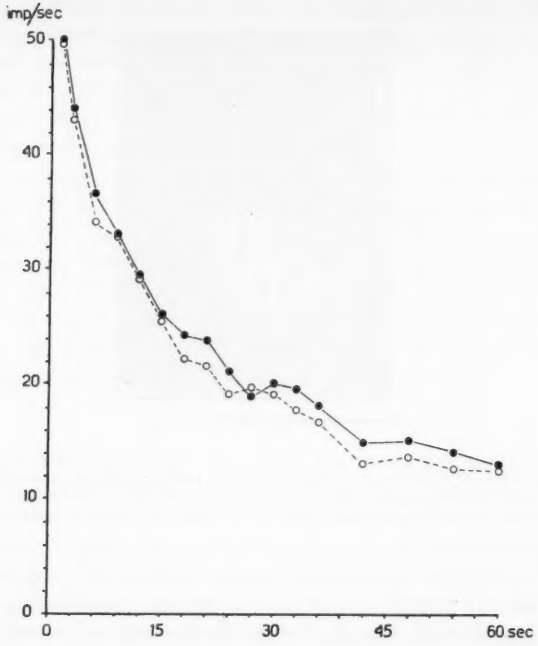

Fig. 20. Average frequency-time curves of interneurones in response to standardized stretch. *Filled circles:* mean values of discharge frequencies of 67 interneurones activated by extensor and flexor groups of ankle joint and flexor group of knee joint. *Open circles:* mean values of discharge frequencies of 22 interneurones activated by triceps surae. *Abscissa:* time in sec from onset of discharge.

In Fig. 20, one curve is made up of points (filled circles) representing the mean value of the response frequencies of all 67 neurones together, the first values being measured 1.5 sec after the onset of the discharge, the others with 3 sec interval. The other curve in the same figure (open circles) represents the separate average frequency-time curve from the 22 experiments on the triceps muscle. As seen from the figure, the curve for triceps alone corresponds well with the curve for the three muscle groups taken all together. In both cases there is an initial fairly exponential frequency decrease for about 24—27 sec, followed by a slower decline.

The turning point — *viz.* the point at which the falling phase of the response frequency turns into a comparatively constant

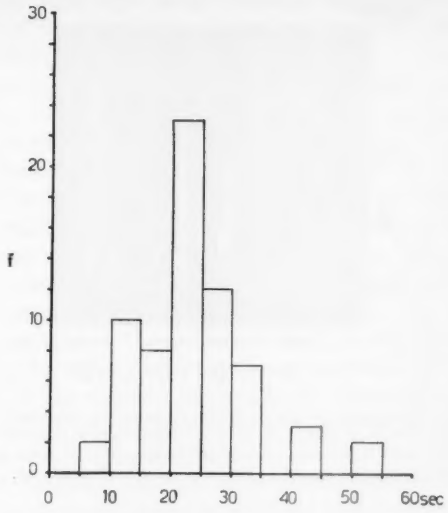

Fig. 21. Frequency distribution of "turning points" of frequency-time curves, obtained from recordings of discharges from 67 interneurones in response to standardized stretch. *Ordinate:* frequency of occurrence; *abscissa:* Time from onset of discharge in 5 sec class intervals.

phase — has been determined in the same way as for the corresponding curve for afferent fibres. As turning point has thus been chosen the point where the impulse frequency of the discharges has fallen to 110 % of their final constant plateau level or, if a plateau level was not reached within 60 sec, the frequency value reached 60 sec after onset of the discharge. In many cases there was a further decline of the response frequency after this period; however, no analysis has been made of these late variations.

The mean value of the turning point thus defined was 24 ± 0.9 sec ($n = 67$, $s = 8$). The frequency distribution of these points is illustrated in Fig. 21, which shows that 82 % of the neurones studied did not reach a fairly steady rate of discharge until after 15 sec and that for 36 % of the neurones the response frequency continued to decline after 25 sec. A comparative analysis of the average curve for the pre- and postsynaptic discharges is presented in the following (p. 40).

Fig. 22. Transient interneurone discharge in response to maintained stretch of quadriceps. Patellar tendon intact. *A*, slow, *B*, rapid flexion of knee joint to 90°; *C*, rapid flexion to 120°; *D*, rapid maximal flexion of knee joint (standardized stretch). Receptors traced to fascia of muscle. *Time* 30 sec.

B. Transient Discharges

As has been mentioned at the beginning of this part, transient postsynaptic discharges in response to maintained muscular stretch have been observed only in about 10 % of the cells studied. When standardized stretch is applied, the transient response usually ceases within 1—5 sec, as judged from the counting rate meter records. As was the case for sustained discharges, the initial impulse frequency of several of the transient discharges can also be shown to depend both on the rate of stretch and the degree of stretch applied to the muscle. This is illustrated in Fig. 22, in which curve *A* shows the irregular, low frequency response to a slow muscle stretch, successively — in 8 sec — rising to a certain submaximal degree of stretch. A more rapid stretch of the muscle up to the same degree of tension results in a twice as high response frequency (*B*). At *C* the same rate of stretch as at *B* has been maintained but the degree of tension has been increased, resulting in a further rise of the response frequency. The last curve (*D*) shows the maximal response frequency from this cell, elicited by rapid stretch up to the maximal physiological degree of stretch. Even this comparatively high initial frequency rapidly falls to zero in spite of the fact that the

muscle was being kept under constant tension, and a new discharge could be evoked only after relaxation and repeated stretch of the muscle.

On three occasions — *viz.* in recordings from three different cells — the receptors setting up the transient discharge could be localized to the fascia of the muscle in question. This was done in the following way. The fascia was exposed by cautious dissection of the skin over the muscle. By a light scratch on the fascia, it was possible to evoke the same type of transient response as previously elicited by stretch of the muscle. After application of a 1 % solution of xylocaine on the scratch-sensitive area the response was immediately abolished. In these cases it seems to be fully established that the transient postsynaptic responses were set up by rapidly adapting fascia receptors (type C receptors according to the classification made by MATTHEWS 1933).

By means of similar experiments using local mechanical stimulation it has been possible, in other cases, to trace the discharges to receptors located in or around joints. It is characteristic that in these cases the response was evoked by changes in the position of the extremities of the animal. In other cases no definite localization of the receptors was possible. It should be mentioned in this connection that some neurones were observed which responded by a typical short discharge of some minutes' duration only after relaxation of a muscle previously stretched.

Various miscellaneous observations on the patterns of these discharges have also been made but so far they are not numerous enough to justify a more detailed presentation.

3. COMMENTS

As demonstrated above, both sustained and transient postsynaptic discharges are set up by maintained stretch of a muscle; in some cases the *transient discharges* could be traced to rapidly adapting receptors. It is, however, not clear whether all transient discharges are induced by activity in rapidly adapting receptors. An alternative is the interpretation (*cf. e. g.* LORENTE DE NÓ 1938 b) that when passing a number of synapses a sustained discharge may quite change in character (duration, frequency) and die out. Conclusive evidence for or against this interpretation has not been obtained in the present investigation. In fact, the

observations made on transient discharges have been included in this paper only complementarily and in order to illustrate that characteristic features of the presynaptic discharge may be observed also in a postsynaptic discharge. The material at hand so far on the transient discharges is not sufficient for a detailed comparison between the two types of discharges.

The principal subject of the following discussion will be the *sustained postsynaptic discharges*. As has been indicated previously, these discharges are thought to be set up in slowly adapting muscular stretch receptors (type A and B receptors according to MATTHEWS 1933). The reasons for this interpretation may be summed up as follows.

The sustained postsynaptic discharges are elicited by passive muscular stretch, and their time-course in response to maintained stretch is, on the whole, analogous to the well-known discharge pattern of these receptors. The variations in the postsynaptic frequency responses due to changes in the rate and the magnitude of stretch applied to the muscle also reflect the well-known variations in the afferent inflow from slowly adapting muscle receptors. By dissection of the musculature it has been possible in a series of experiments to localize the afferent source of this type of postsynaptic activity to the belly of the muscle.

In most of the experiments no attempts have been made to distinguish between muscles and tendon organs; both types of receptors are likely to influence the interneurones studied. It must also be taken into account that a certain neurone may be influenced both by excitatory and inhibitory afferent activity from one and the same muscle (*e. g.* muscle spindles and tendon organs respectively). In some cases a discharge set up by stretch of a muscle could be seen to cease when the tension of the muscle was increased. A possible explanation may be that initially only the low threshold muscle spindles are active but that, with increasing tension, a discharge is set up also in the tendon organs which may have an inhibitory influence on the postsynaptic neurone. However, there may be other interpretations; thus, for example, a higher rate of loading of a muscle may result in a decline also of the peripheral afferent inflow (MATTHEWS 1933).

Spontaneous activity. Using surface electrodes in experiments on the cat's spinal cord, it has been established that a certain "spontaneous activity" is present (MARK and GASTEIGER 1953; for references see TEN CATE 1950). Thus, it is only to be

expected that a resting discharge should be observed in some of these interneurones. In the system of interneurones with proprioceptive connections studied in this work it has been possible, however, in 60 % of the neurones to localize the origin of the resting activity to spontaneous, or experimentally induced, receptor activity in the muscle. In about 40 % of the neurones no definite peripheral source in the hindlimbs could be found. It has, however, been demonstrated that an inflow from pain receptors (KOLMODIN and SKOGLUND 1957) as well as peripheral activity from other parts of the animal, particularly the forelimbs (LLOYD 1942; KOLMODIN and SKOGLUND 1954 b) converge on single units in these spinal cord segments. Nor should other end organs, *e. g.* temperature receptors, be neglected as a possible peripheral source of the resting activity, even though there is so far no positive evidence of a convergence of this type in the neurones studied. The present investigation has not aimed at a full analysis of the origin of the spontaneous activity. Of special interest is, however, that a considerable part of the resting activity in the neurones studied could be traced to peripheral activity.

An evidence of the variations existing in the activity level of these units engaged in the proprioceptive spinal mechanism is the fact that in one and the same experiment have been found initially silent neurones and neurones with a resting activity induced from a peripheral source, both types excitable from the same muscle.

Range of discharge frequency. As reported earlier (KOLMODIN and SKOGLUND 1954 b; FRANK and FUORTES 1955), interneurones in the lumbar region have been found to respond to natural stimulation by discharges at rates up to 200/sec. Using the standardized stretch stimulation applied in this work, the initial response frequency of the interneurones studied has, as a rule, varied between 20 and 100 per sec, but frequencies of 150—200 per sec and even higher have also been observed. There is reason to believe that higher frequencies may be found in intact animals. Considerably higher frequencies have been observed in the muscle spindle discharges in animals with an intact efferent gamma system (ELDRED, GRANIT and MERTON 1953) and, consequently, the postsynaptic frequencies are also likely to be higher in such animals.

Using electrical stimulation it has been shown that these interneurones are able to discharge at rates above 600/sec, at least

for very short periods of 10—100 msec (WOODBURY and PATTON 1952; ECCLES, FATT and LANDGREN 1954, 1956; FRANK and FUORTES 1955; MCINTYRE, MÄRK and STEINER 1956).

An interesting fact is that the discharge frequencies observed in interneurones during standardized stretch are of the same order of magnitude as those recorded from afferent fibres, whereas, on the other hand, they are higher than the frequencies induced by natural stimulation in motoneurones (ADRIAN and BRONK 1929; *cf. e. g.* also ALVORD and FUORTES 1953).

Judging from the work of LAPORTE and LUNDBERG (1956), the impulse frequency in single elements of the dorsal spinocerebellar tract induced by muscular stretch seems to be lower than that observed in recordings from interneurones in the grey matter, but the significance of these differences cannot be judged until more detailed data are at hand on the range of the frequency variations in ascending tracts.

4. COMPARISON BETWEEN SUSTAINED AFFERENT AND POSTSYNAPTIC DISCHARGES

Factors determining the postsynaptic discharge rhythm. As shown in the records of action potentials, Figs. 7 and 12—14, the most striking difference between a discharge in an afferent fibre and in an interneurone is the marked irregular spacing of the impulses in the interneurone response. An irregular impulse spacing in postsynaptic neurones activated via muscular stretch receptors has previously been observed by COOPER, DANIEL and WHITTERIDGE (1953) in their studies on the central pathways of the eye muscle afferents in the goat, and also, *e. g.*, by KOLMODIN and SKOGLUND (1954 b), FILLENZ (1955) and FRANK and FUORTES (1956). The spacing of the impulses may be completely irregular, or the impulses may show a tendency towards grouping which is also reflected in more or less irregular variations in the frequency curve of the interneurones as recorded by the counting rate meter. It is noteworthy that in the present investigation this irregular spacing has been observed in all interneurones responding to muscular stretch by a sustained discharge, whereas afferent fibres activated in the same way have never shown any obvious irregularity.

If one assumes that a 1 : 1 or else constant relationship exists between the number of afferent and postsynaptic impulses, it may seem remarkable at first sight that there should be such a difference in the pre- and postsynaptic activity in regard to their regular rhythm. However, in spite of the regular frequency in the single afferent fibres there are, as a matter of fact, several reasons why the interneurone discharge may be expected to be irregular. In the first place, there is reason to presume that several afferent fibres from one muscle converge on one and the same interneurone (*cf.* Fig. 26), and it is most likely that the impulses are travelling in and out of phase in different fibres. If certain afferent fibres have no direct connections with the interneurone studied but converge on it via internuncial neurones, there are of course still more chances that the total inflow of impulses reaching the interneurone should be irregular. The possibility of reverberating activity in closed internuncial chains must also be taken into account.

Furthermore, there is a great deal of evidence pointing to a transformation of the presynaptic impulse pattern during the course of the synaptic processes to a completely new pattern largely determined by the inherent rhythmical properties of the postsynaptic membrane. From analyses of electrotonic root potentials, Barron and Matthews (1938) concluded that, as a result of a natural afferent inflow, slow depolarization processes occur at primary terminations inducing potential changes of similar slow time characteristics in the postsynaptic membrane. For motoneurones the discharge frequency was found to be dependent on the level and rise of the slow electrotonic potential changes. By means of intracellular recording of the actual membrane potential changes occurring in spinal neurones during natural stimulation Kolmodin and Skoglund (1957) were able to show that the postsynaptic discharge frequency is related to the level of polarization but also that rather complex mechanisms, involving oscillatory behaviour of certain fractions of the membrane potentials, determine the repetitive firing of naturally activated central neurones.

Finally, when considering the factors determining the postsynaptic discharge rhythm, the possibility that interneurones activated via muscle stretch may be influenced simultaneously both by excitatory and inhibitory fibres should also be taken into account (*cf.* Laporte and Lundberg 1956).

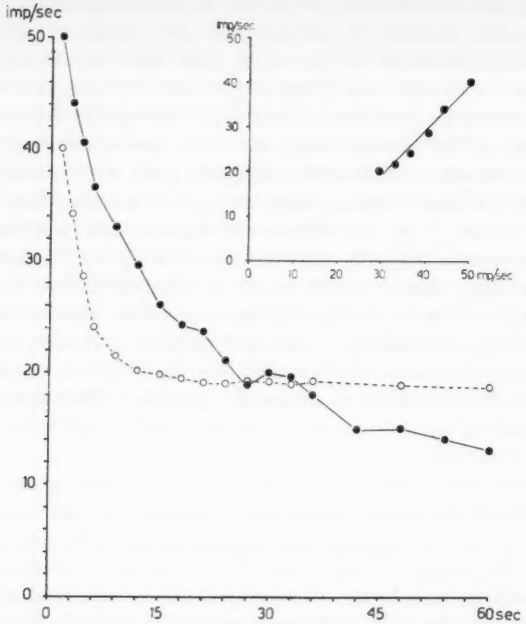

Fig. 23. Comparison of average frequency-time curves of afferent fibres and interneurones. *Open circles:* curve of 75 afferent fibres, from Fig. 8. *Filled circles:* curve of 67 interneurones, from Fig. 20. *Abscissa:* time in sec after onset of discharge. *Inset figure* shows relation between average afferent (*ordinate*) and interneurone (*abscissa*) discharge frequencies during initial fall of curves (first 12 sec).

In view of the large number of varying factors thus influencing the postsynaptic discharge frequency, it is rather surprising that the pre- and postsynaptic frequency-time curves have so many features in common. A comparative analysis of the two curves and some comments on their various phases should be of interest.

Comparison of pre- and postsynaptic frequency-time curves. In Fig. 23, the curves for all muscle groups from Figs. 8 and 20 are plotted together in order to compare the absolute frequency values of fibres and interneurones at various time intervals, whereas, in Fig. 24, the same curves are plotted with relative frequency values in per cent of the initial maximum frequency recorded.

As appears from Fig. 23, there is a rapid decline of the impulse frequencies in both curves for the first 12 sec after onset of the discharge. The relation between the afferent and the internuncial frequency during this initial fall of the curve is shown in the inset figure; it appears that in this phase there is, roughly, a direct proportionality between the frequency values.

It should be realized that the frequency transformation represented by these curves is taking place in what may be called "*an average interneurone*", and that the results are not directly transferable to processes in the single interneurones; also for other reasons their general significance has to be judged with great caution. Although the observations made have been based on a continuous physiological frequency variation, the relationship between pre- and postsynaptic frequencies has only been studied in successively *falling* frequencies; besides, the processes studied have been followed for a comparatively long time, so that various adaptation and accommodation phenomena may very well have occurred.

It also appears from Fig. 23 that, when the afferent average curve has reached a fairly constant level, after 13.5 sec, there is still a continued frequency fall in the interneurone curve, at first rather rapid but successively slowing down in the last part of the curve. A detailed comparison of these late phases of the curves can best be done by means of the relative frequency curves in Fig. 24. It appears that in the interval from 14 to 24 sec, when the afferent impulse frequency declines by about 2 %, the postsynaptic frequency is reduced by about 18 %; after that period it declines more slowly.

This feature of the postsynaptic curve may be said to represent an *adaptation* of the average interneurone to the comparatively constant activation set up by the total afferent inflow during this period.

It should be pointed out that the differences in frequency in the two curves during the late phase are too great to be interpreted as being a mere chance. As shown in Fig. 9, 95 % of the afferent fibres have reached their constant discharge level after 25 sec, whereas in 36 % of the interneurones the discharge frequency continues to decline after that time (Fig. 21).

The mean values for the turning points of the frequency-time curves for the fibres (p. 24) was 13.5 ± 0.8 ($n = 75$, $s = 7$) and for the interneurones (p. 35) 24 ± 0.9 ($n = 67$, $s = 8$). The

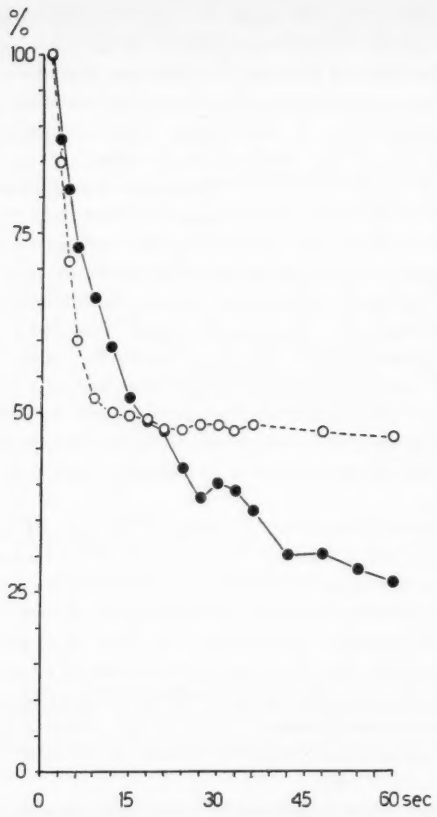

Fig. 24. Same average frequency-time curves as in Fig. 23 plotted with relative frequency values in per cent of initial maximum frequency recorded. *Open circles:* curve of 75 afferent fibres. *Filled circles:* curve of 67 interneurones.

difference betweeen the two means was 10.5 ± 1.5 ($t = 4.6$, $df = 140$). The difference was thus highly significant ($p < 0.001$).

It is doubtful whether a corresponding significant adaptation process takes place during the initial rapidly falling phase of the curve. However, the slightly slower decline of the postsynaptic average frequency-time curve in relation to the presynaptic one suggests that a mechanism of this kind may be involved.

II

Convergence Patterns of Interneurones influenced from the Ipsilateral Hindlimb

Introductory remarks.

As already mentioned, interneurones with proprioceptive connections can be activated from more than one afferent source. In this part of the paper a description will be given of the various patterns of afferent connections — both proprioceptive and exteroceptive — observed in interneurones of this type. This survey will give an idea of how the convergence system of interneurones involved in a proprioceptive spinal mechanism may be organized.

The present analysis has been limited to interneurones that can be influenced from receptors located in the *ipsilateral hindlimb*. A number of neurones with both ipsi- and contralateral afferent connections have been found (*cf.* KOLMODIN and SKOGLUND 1954 b) but these have not been included in the present investigation.

The inhibitory and excitatory sources converging on a given unit were localized in the following way. First of all the various sources activating the neurone were traced, one after the other, by stretch as well as by pressure, touch and pain stimulation. Then constant stretch was applied to one of the muscle groups influencing the neurone, and the hindlimb was tested again with natural stimulation of other proprioceptive and exteroceptive sources. Then, in turn, constant stretch was applied to all muscle groups likely to influence the neurone, one group after the other, and the procedure was repeated. The various sources that had proved to have an excitatory or inhibitory influence on the neurones were thus tested, as far as possible, in all conceivable combinations. The contralateral hindlimb was roughly tested in order to reveal afferent influence from this source, and any neurones with an activation of that kind were excluded.

As index of the activity induced in this way, the discharge evoked in an initially silent neurone, or the increase or decrease of an already existing discharge frequency has been chosen. No separate analysis has been made of initially silent neurones and

neurones with a resting activity. It should be pointed out in this connection that the inhibitory action of certain sources was not revealed until simultaneous stimulation of two different sources was applied. Thus, in many initially silent neurones, stretch of a muscle group set up a postsynaptic activity that could be inhibited by simultaneous stretch of another muscle group.

In the description of the various convergence patterns it is understood that an afferent source with an excitatory or inhibitory effect on the discharge evoked from another source is considered to establish a functional connection with the neurone in question, whether this neurone is influenced directly or via intercalated neurones.

The total number of neurones studied in these attempts at an analysis of their afferent connections has been about 200. However, many of them have been excluded from the present description, for varying reasons. For example, the localization procedure has to be carried on for some length of time and could only be carried through in neurones where the recording could go on for a sufficiently long time. In other cases it has been difficult to determine the exact position and modality of the excitatory and inhibitory sources; for instance, many neurones have been excluded because of difficulties sometimes encountered to distinguish between muscle receptors, joint receptors and pressure receptors (*e. g.* in the pad). A number of neurones have not been included because they have shown deteriorating resting potentials or other signs of injury.

The present description is based on 89 interneurones which have been sufficiently analysed to merit a description. The neurones have been divided into different groups, according to whether they have been excitable from one, two or three functionally different muscle groups. Each group includes neurones in which only excitatory effects could be elicited as well as neurones in which both excitatory and inhibitory effects have been observed. In order to make it easier to follow the description, the various convergence patterns observed have been depicted schematically in Figs. 25, 27—29. The position of the electrode tip during the different recordings is also shown in the figures. All recording points have, however, not been indicated, because in some cases the electrode has been dislocated after the end of the experiment and in other cases the histological sections were not satisfactory.

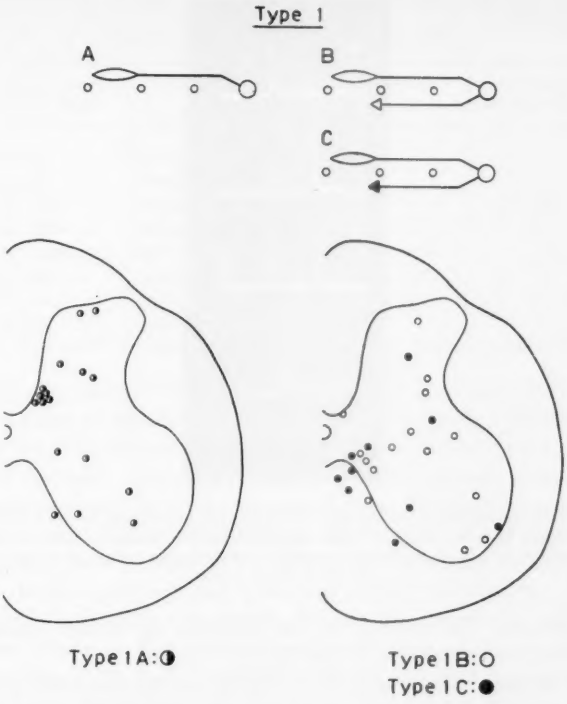

Fig. 25. *Top of figure.* Diagram illustrating patterns of convergence of interneurones which could be influenced from one muscle or group of synergistic muscles (type 1). *A*, *B* and *C* are schematic drawings of ipsilateral hindlimb, small open circles indicating — from left to right — ankle, knee and hip joint. Large open circle represents interneurone, ovals indicate muscle groups, triangles one or more exteroceptive sources, open symbols excitatory and filled inhibitory action.

Bottom of figure. Schematic cross section of lumbar spinal cord indicating the recording sites of various convergence types of interneurones.

1. CONVERGENCE PATTERNS COMPRISING ONE MUSCLE GROUP

A. Neurones Activated by only one Muscle Group

The schematic drawing in Fig. 25 (type 1 *A*) indicates the group of neurones activated by one muscle group solely. Nineteen neurones of this type have been found with the following muscle groups as activating sources: the quadriceps, biceps-semitendinosus, triceps surae, tibialis anterior, plantaris and extensor digitor-

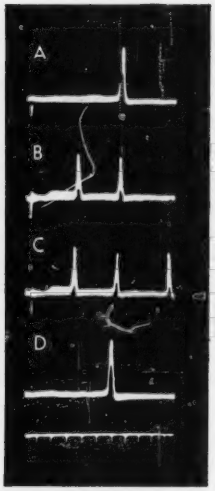

Fig. 26. Intracellular responses from dorsal horn interneurone, *A*—*C*, to electrical stimulation of increasing intensities applied to nerve of medial head of gastrocnemius, and *D*, to maintained stretch applied to the tendon of the muscle. See text. *Time* 1 msec.

um groups. The position of the electrode tip in the various recordings is also shown in the figure.

In experiments on some of these muscles it has been possible to localize the activating source more exactly to a certain part of the muscle. Thus, for example, in one case it could be traced to the medial head of gastrocnemius from the observation that the interneurone discharge in response to muscle stretch was uninfluenced by cutting the nerve to the lateral head and disappeared only after severing of the medial nerve. Also when electrical stimulation was used to set up afferent volleys in the two nerves only those mediated by the medial nerve were found to be effective.

In agreement with WOODBURY and PATTON (1952), KOLMODIN and SKOGLUND (1954 b) observed in experiments with electrical stimulation of dorsal roots that in many neurones the number of spikes in the repetitive response could be graded by varying the stimulus strength. This observation could be explained most likely by a convergence of several fibres of the stimulated root on one and the same interneurone.

In order to elucidate the question whether interneurones activat-

TABLE I

Afferent connections of type 1 B neurones

Excitatory proprioceptive source	Excitatory exteroceptive sources	Number of neurones found
Quadriceps	pad pressure	4
Quadriceps	touch of vaginal introitus	1
Triceps surae	sural nerve, el. stimulation	4
Triceps surae	pad pressure	3
Triceps surae	pad pressure + hair between pads	2
Tibialis ant.	pad pressure	3
Tibialis ant.	pad pressure + hair between pads	1
Flexor dig.	pad pressure	2

ed only by muscular stretch receptors had a convergence of this kind, a series of experiments was done, one of which is illustrated in Fig 26. The neurone responded to stretch of the muscle by a typical sustained discharge. A single action potential of such a discharge is shown in *D*. The nerve to the muscle was then cut and placed on stimulating electrodes. As the strength of the electrical stimulation was increased, there was an increasing number of spikes in the discharge and a reduced latency of the first spike (records *A*—*C*). The experiment favours the view that several afferent fibres in this muscle nerve converge on the neurone (*cf.* ECCLES, FATT and LANDGREN 1956); it is a fact that at least one of these fibres transmits impulses evoked by stretch.

B. Neurones Activated by one Muscle Group in Combination with Exteroceptive Afferent Sources

Neurones with excitatory properties solely

Twenty neurones have been found with a convergence pattern like that drawn schematically in Fig. 25 (type 1 *B*). A single neurone in this group could be activated (*i*) by stretch of one muscle group as well as (*ii*) from one, in some cases two, exteroceptive sources. The position of the neurones is shown in the figure.

The various muscle groups and exteroceptors serving as activating sources in the single cases are shown in Table 1. As appears from the table, the most common exteroceptors in this type of convergence pattern were pressure receptors in the pad. In the cases where natural stimulation revealed more than one extero-

TABLE II

Afferent connections of type 1 C neurones

Excitatory proprioceptive source	Excitatory exteroceptive source	Inhibitory exteroceptive sources	Number of neurones found
Gastroc. nerve, el. stimulation		sural nerve, el. stimulation	1
Gastroc. nerve, el. stimulation		pad pressure	2
Triceps surae		sural nerve, el. stimulation	2
Triceps surae		pad pressure	3
Quadriceps		pad pressure	2
Quadriceps	pressure of tail	pad pressure + pain of tail	1
Tibialis ant.		pad pressure	2
Tibialis ant.		pain of pad and tail	2
Tibialis ant.	hair on foot	pressure of tail	1

ceptive source, this was found to be the hair between the pads. The combination of quadriceps stretch and touch of vaginal introitus in one experiment suggests, however, that exteroceptive inflow from other areas may also converge on this type of interneurone. It should be mentioned that a couple of neurones (not included in Table 1, owing to incomplete analysis; they had probably also contralateral connections) were found to respond to stimulation of the hair of the tail and, in some cases, to touch of the hair over the inside of the thigh.

Neurones with excitatory and inhibitory properties

In sixteen neurones of type 1 both excitatory and inhibitory effects could be evoked. The convergence pattern of these neurones is indicated in Fig. 25 (type 1 *C*) and their position is shown in the same figure. They could be activated from one muscle and, in addition, from one exteroceptive source in two cases. The inhibitory effect was elicited from one, in some cases from two different exteroceptive sources. The different muscle groups and exteroceptors serving as sources of activation and inhibition in the various cases are presented in Table II. As shown in the table, the inhibitory source in natural stimulation has in most cases been traced to the pad. A similar localization was obtained when different afferent nerves were tested with electrical stimulation.

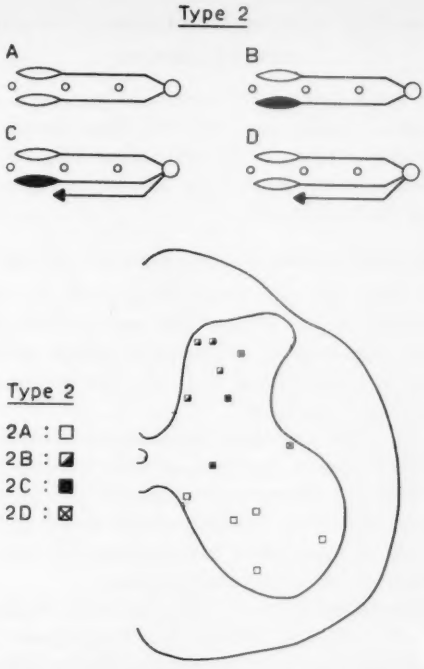

Fig. 27. Diagram illustrating patterns of convergence and position of interneurones which could be influenced from two different muscle groups acting as antagonists at the same joint (type 2, see text). Symbols for upper diagrams as in Fig. 25.

The table does not include any neurones activated by the flexor group of the knee joint (biceps-semitendinosus). Actually in some cases neurones were observed whose activating receptors were probably located in this muscle group. The observations made on this group are, however, not sufficiently analysed for a presentation in this work.

No neurones activated by an exteroceptive source and inhibited by muscle stretch were found. A pattern of afferent connections of this type may, however, very well exist in view of the fact that neurones with both contralateral and ipsilateral afferent connections have been found, in which the activity from an exteroceptive source was inhibited by stretch of a muscle.

2. CONVERGENCE PATTERNS COMPRISING TWO DIFFERENT MUSCLE GROUPS

In twenty-seven neurones a convergence pattern comprising two different muscle groups was observed. These muscle groups have been antagonists acting at the same joint (Fig. 27), synergists acting at neighbouring joints (Fig. 28) and antagonists acting at neighbouring joints (Fig. 29).

Antagonistic muscle groups acting at the same joint

Seventeen neurones were found that could be influenced by two antagonistic muscle groups. The convergence patterns thus observed have been divided into four sub-groups, which are shown schematically in Fig. 27 (type 2 *A*—*D*). The position of the cells also appears from the figure.

In seven of these seventeen neurones only excitatory effects could be evoked (type 2 *A*). Three of them were activated by the quadriceps and the biceps-semitendinosus groups, whereas four could be activated both by the triceps surae and the tibialis anterior groups. None of these seven neurones could be activated by stimulation of an exteroceptive source.

In ten different neurones of this type both excitatory and inhibitory effects could be elicited. The convergence patterns observed are indicated in Fig. 27 (type 2 *B*—*D*). Six different neurones were found with a convergence pattern of type 2 *B*. The discharge evoked by stretch of one muscle group could be inhibited by simultaneous stretch applied to the antagonistic muscle group. Thus for example, neurones were observed in which the activity evoked from the triceps surae was inhibited by the tibialis anterior group and *vice versa*. In the same way, neurones excitable by the quadriceps group were inhibited by the biceps-semitendinosus group; in one neurone an inversed pattern was found.

A somewhat different type of pattern was found in two neurones (type 2 *C*); they had a muscular convergence pattern similar to that in *B* and, in addition, could be inhibited by stimulation of an exteroceptive source. In both cases the activating muscle group was the quadriceps which was inhibited by its antagonist and by pressure of the ipsilateral pad.

It is of interest that all neurones, which could be influenced in this way by antagonistic muscle groups, were situated in the dorsal horn. In one experiment, three of the neurones which could be

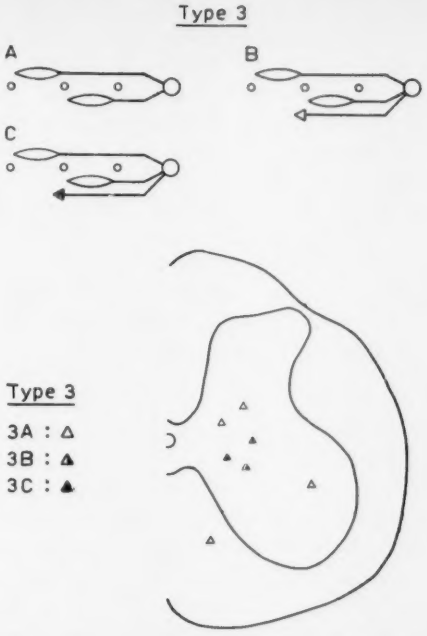

Fig. 28. Diagram illustrating patterns of convergence and position of interneurones which could be influenced from two different muscle groups acting as synergists at neighbouring joints(type 3, see text). Symbols for upper diagrams as in Fig. 25.

reciprocally influenced by the extensors and flexors of the ankle were found along the same needle track only about 50 μ from each other.

Two neurones have been observed with a convergence pattern designated type 2 *D*. One of them was influenced by the extensors and flexors of the knee joint, the other one by those of the ankle joint; in both cases the inhibitory source was pressure on the pad. Again, one of these neurones was found in the dorsal horn. (The position of the other neurone could not be exactly determined for technical reasons.)

Synergistic muscle groups acting at neighbouring joints

Among the neurones with convergence patterns like those shown in Fig. 28, six proved to be purely excitatory. Four neuron-

es of the type 3 *A* in the figure could be activated by the extensor group of the knee (the quadriceps group) and that of the ankle joint (triceps surae) and one neurone by the corresponding flexors (biceps-semitendinosus and the tibialis anterior group). A neurone with a similar type of afferent connections (type 3 *B*) could also be activated by pressure on the ipsilateral pads (both the big and the small pads).

Two neurones have been found with both excitatory and inhibitory properties (type 3 *C*). In both cases the activating muscle groups were extensors (quadriceps and triceps surae), and the inhibitory effect on the discharge elicited from these sources was evoked by pressure on the large ipsilateral pad. The position of the neurones is shown in the diagram Fig. 28.

Antagonistic muscle groups acting at neighbouring joints

Five different neurones were found that could be activated from antagonistic muscle groups acting at neighbouring joints (Fig. 29, type 4). Two neurones of type 4 *A* were observed with only excitatory properties. Both neurones could be activated by stretch of the quadriceps and tibialis anterior muscle groups. Besides one of them was activated by hair stimulation between the small pads, the other by pressure on the pad. Three neurones of type 4 *B* could be activated by stretch of quadriceps, and the activity thus set up could be inhibited by simultaneous stretch of the tibialis anterior group. No exteroceptive source was found in any of these three neurones. The position of the neurones is given in the diagram.

3. CONVERGENCE PATTERNS COMPRISING THREE DIFFERENT MUSCLE GROUPS

Five neurones which could be influenced from three different muscle groups have been found (Fig. 29, type 5). The position of the neurones is shown in the same figure.

In all these cases the muscle groups involved were the quadriceps, the triceps and the tibialis anterior. In two neurones of type 5 *A* stretch of either of these muscle groups or pressure of the ipsilateral pad could evoke a postsynaptic activity, whereas in two neurones of type 5 *B* the activity evoked from the quadriceps could be inhibited by simultaneous stretch of either the extensors or the flexors of the ankle.

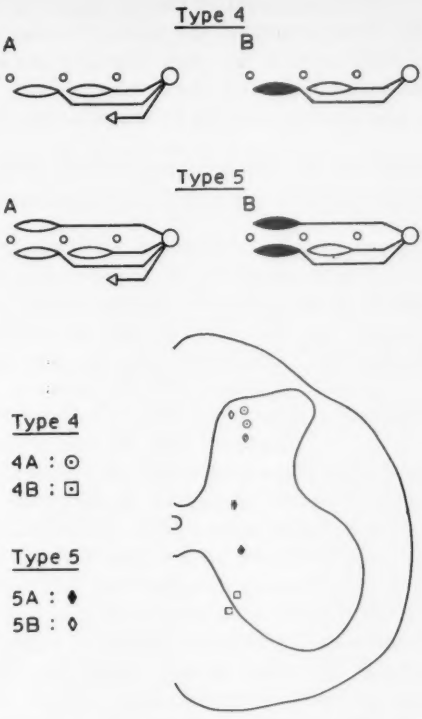

Fig. 29. Diagram illustrating patterns of convergence and position of interneurones which could be influenced from two different muscle groups acting as antagonists at neighbouring joints (type 4, see text) and of interneurones which could be influenced from three different muscle groups (type 5, see text). Symbols for upper diagrams as in Fig. 25.

Miscellaneous observations

Three neurones were found showing patterns of convergence, the functional organization of which was different from the types just described. One of these neurones could be activated by stretch of either the quadriceps or the triceps muscles. The postsynaptic activity set up by triceps stretch could be inhibited by simultaneous pressure applied to the pad, whereas the activity set up from the quadriceps muscle was uninfluenced by pad pressure (*cf.* neurone *A* in Fig. 39). The two other neurones were spontaneously

active. They could be activated by stretch applied to the extensor as well as the flexor group of the ankle. In the one neurone pressure of a small pad, in the other touching the hair between the pads was found to inhibit the resting activity but exerted no effect on the activity induced by muscular stretch.

III

Interneurone Responses to Separate and Simultaneous Stimulation of Different Afferent Sources

In the intact animal, the various afferent sources involved in the convergence patterns of the interneurones described above may under certain conditions be activated separately, and under other conditions in varying combinations. Thus, for example, in the flexor phase of a leg movement there may be an afferent inflow from a certain muscle or muscles, whereas in the opposite phase, when the foot reaches the ground, afferents from pressure receptors in the pad are also involved.

It should therefore be of interest to make a systematic investigation of the postsynaptic discharges during (*i*) separate and (*ii*) simultaneous activation of the various sources influencing a certain neurone. In the following will be presented some typical results from studies of this kind, selected from a group of about twenty interneurones, a complete analysis of which could be carried out under satisfactory recording conditions. Before the analysis could be made, the convergence pattern had to be determined in each case, and this being a time-consuming procedure, signs of deterioration were apt to appear in some neurones which therefore had to be excluded.

The following description includes only the features most commonly observed and which could be supposed to be generally valid; many units exhibiting special features, interesting as such but only occasionally observed, have thus been excluded.

1. COMPARISON OF THE RESPONSES TO SEPARATE STIMULATION OF VARIOUS PERIPHERAL EXCITATORY SOURCES

In the studies of the responses in a certain neurone elicited by various afferent sources, only *physiologically comparable* stimuli have been used, *viz.* either (*i*) standardized stretch or (*ii*) a stimulation evoking a maximal postsynaptic frequency response. Whichever type of stimulation was being used, the frequency-time curves of the discharges set up in an interneurone by separate stimulation

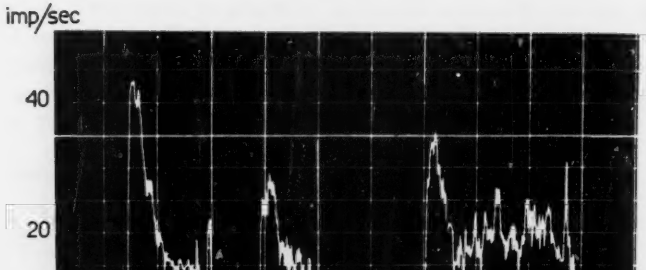

Fig. 30. Frequency-time curve obtained from ventral horn interneurone activated by standardized stretch applied, separately, to the cut tendon of *A*, triceps surae, *B*, quadriceps, and *C*, by pressure of the large pad of the same limb. *Solid line:* duration of stimulus. *Dotted lines in curve* indicate break in recording. *Time* between vertical lines 30 sec.

of various sources were, as a rule, found to differ considerably both in regard to absolute frequency values and decay of the frequency and in regard to frequency fluctuations, all depending on the source activated.

An experiment illustrating these observations is shown in Fig. 30. The neurone studied could be activated by two different muscle groups (the extensor groups of the knee and ankle joints) and also from pressure receptors of the pad. *A* shows the response to stretch of the Achilles tendon corresponding to maximal flexion of the ankle joint; *B* shows the response after loading of the quadriceps tendon corresponding to maximal flexion of the knee joint. In fact, the frequency responses obtained in these cases represent the maximal responses elicitable from these muscles. In many other neurones the response frequency could, as a rule, be increased by a further loading of the muscle beyond these "physiological" limits. The general features of the two discharge patterns in response to maintained muscular stretch are the same as described in part II above; as appears from Fig. 30 they are characterized by a comparatively high initial frequency decreasing at a fairly rapid rate to a slowly adapting irregular frequency. In some

respects, however, the two responses differ. There is, for example, a marked difference in the initial frequency even though this may in part be explained by unintentional variations in the application of standardized stretch. There are, however, obvious differences in the decay of the frequency-time curve and the final frequency level. Most striking are perhaps the very pronounced fluctuations in frequency when the quadriceps muscle is subjected to stretch.

As mentioned above, the responses illustrated in Fig. 30 also represent the maximal frequencies elicitable in the neurone via these sources. Whether standardized stretch evoked the maximum frequency response or not, there were, in most cases, significant differences in the frequency-time curves, when different muscular sources were being activated.

When comparing the responses in a certain cell elicited by receptors of *different sensory modality*, an equal basis for the comparison could best be obtained by using stimuli always evoking the *maximal* frequency response.

As mentioned above the neurone described in Fig. 30 could also be activated by pressure receptors in the pad. The curve in Fig. 30 *C* represents a typical frequency response elicited by constant pressure of the pad, as will be discussed in detail in another paper (KOLMODIN and SKOGLUND 1957). It is obvious that the time-course and the frequency pattern of the curve differ from those elicited by muscular stretch.

In many neurones, the differences observed in the maximal frequency responses elicited by stimulation of sources of different modality were still more striking than those illustrated in Fig. 30. Fig. 31 shows the response discharge from a neurone elicitable from only one muscle group and a pad. There is a marked difference in the responses from the two sources, both in the initial frequency and in the rate of decline. It is of interest that also in one and the same neurone there is a varying degree of irregularity of the response frequency.

2. DISCHARGES OF INTERNEURONES IN RESPONSE TO SIMULTANEOUS STIMULATION OF TWO DIFFERENT PERIPHERAL SOURCES

Simultaneous stimulation of two afferent sources converging on a certain neurone may result in an increase (summation) or a decrease (inhibition) of the response frequency as compared with

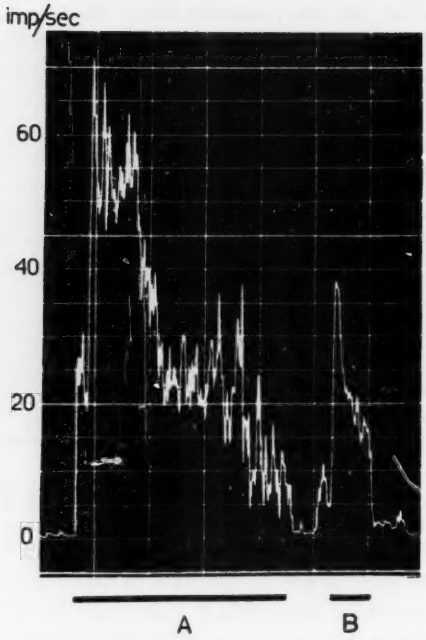

Fig. 31. Frequency-time curves illustrating different types of discharges of an interneurone in the intermediate region in response to: *A*, standardized stretch of triceps surae; and *B*, pressure (400 g) applied to the large pad of the same limb (maximal response). Note stepwise decline and marked irregularity of the discharge frequency in *A*. *Solid line:* duration of stimulus. *Time* 30 sec.

separate stimulation of each of these sources. As has been shown in the description in part II, only excitatory effects could be elicited in one group of neurones, whereas both excitatory and inhibitory effects could be evoked in another group. Summation effects have mostly been studied in neurones with purely excitatory properties.

A. *Postsynaptic discharges in response to simultaneous stimulation of two excitatory sources*

In 27 of the 56 neurones in which purely excitatory effects could be evoked, it has been possible to study the effect of simultaneous stimulation of two different afferent sources. Although in 8 of the neurones tested no definite summation effects could be

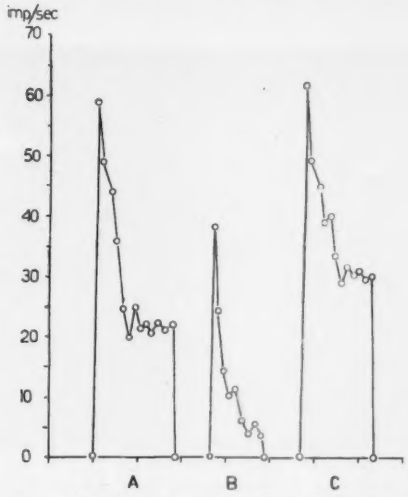

Fig. 32. Spatial summation. Graphs of frequency of response to standardized stretch of: *A*, biceps-semitendinosus muscle; *B*, quadriceps muscle, and *C*, both muscle groups simultaneously. *Time* 30 sec.

observed, in 19 of the neurones the result was an increase of frequency, both in the initial and in the later phase of the response. This is illustrated in Figs. 32 and 33 (*cf.* also Fig. 34). In the experiment shown in Fig. 32 the neurone could be activated by two different muscle groups acting as antagonists at the same joint. *A* is the response frequency after maintained maximal extension of the knee joint. By applying slight pressure, the receptors in *A* were traced to the belly of the lateral head of the biceps muscle. The response from this muscle had a low threshold to stretch. *B* shows the corresponding curve for stretch applied to the tendon of the quadriceps muscle, the amount of stretch corresponding to full flexion of the knee joint. There was a high threshold response and the receptors were traced to the distal part of the muscle in the vicinity of the patellar tendon. In *C* both muscle groups have been stretched simultaneously, which resulted in an increase of the response frequency, both in the initial and in the later phase. The position and the threshold values of the receptors activated indicate that muscle spindles may have been active in the quadriceps and tendon organs in the biceps-semitendinosus. Conclusive evidence of the type of receptors involved could not be obtained in this case, nor in other cases studied.

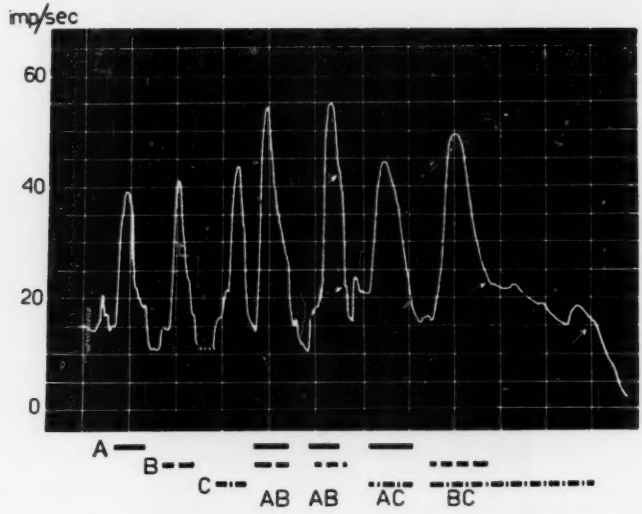

Fig. 33. Spatial summation as seen in frequency-time curve obtained from single interneurone. Application of a load of: *A*, 400 g to tendon of triceps muscle, and *B*, 500 g to tendon of quadriceps muscle; *C*, pressure corresponding to 400 g applied to the pad. *Solid and dotted lines:* duration of stimulus in combinations described. Integrating time constant 5 sec.

Fig. 33 illustrates a neurone with another convergence pattern. This neurone could be activated by two different muscle groups acting as synergists at different joints, the triceps surae (*A*) and the quadriceps (*B*), and by pressure on the ipsilateral large pad (*C*).

As in the preceding experiment, the different excitatory sources were at first activated separately (*A* 400 g, *B* 500 g, *C* 400 g). As is seen in *AC*, *AB* and *BC*, a combination of two of the different sources results in an increase of the maximal frequency and of its duration. At the first arrow in *AB* the quadriceps was unloaded resulting in a rapid fall of the impulse frequency to about the same level as is reached by separate loading only of the tendon of the triceps surae after the same period of time (*cf. A*). In the combination *BC* (the quadriceps and the ipsilateral large pad) the first arrow marks the unloading of the quadriceps. In this case the impulse frequency is slowly adapting to a level corresponding to the adapted level in *C*. At the second arrow the pad also is unloaded which results in a cessation of the discharge in about 15 sec.

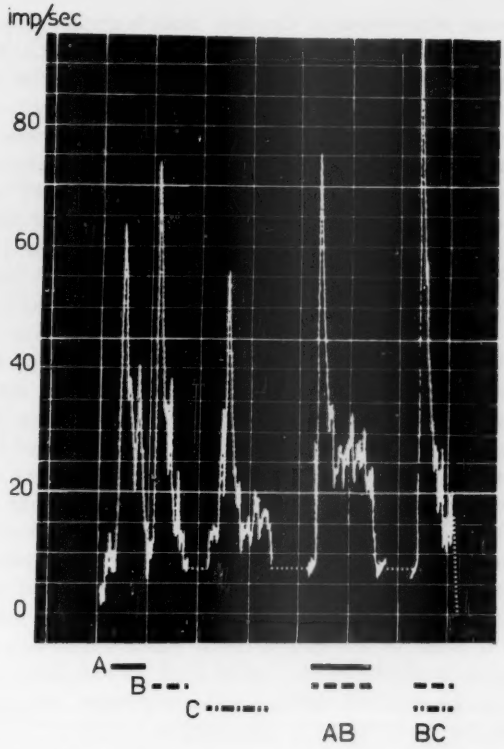

Fig. 34. Different kinds of spatial summation as seen in frequency-time curve obtained from one and the same interneurone. Standardized stretch applied to: *A*, quadriceps, *B*, triceps and *C*, tibialis anterior group. *Solid and dotted lines:* duration of stretch in combinations described. *Dotted lines in curve* indicate break in recording. *Time* 30 sec.

As shown in these experiments, simultaneous stimulation of two different afferent sources may result in a significant increase of both the initial and the later phase of the discharge.

In the study of these summation effects, however, it was also observed that in some cases simultaneous stimulation of two afferent sources resulted in an increase *either* of the initial *or* of the later phase of the response frequency. In some cases, both types of summation effects were observed in one and the same neurone. An example is shown in Fig. 34; the neurone could be activated from three different muscle groups, *A*, *B* and *C*. The effect of

simultaneous stimulation of the two muscle groups *A* and *B* is illustrated at *AB*. There was no increase in the initial response frequency whereas the frequency of the later part of the discharge has increased. On the other hand, a combination of the two synergists *B* and *C* results in a marked increase of the initial response frequency, while there is no summation effect in the later part of the response. The combination *AC* was also tested and, judging from the discharges heard in the loud-speaker, there was a summation effect also in this case; however, before the summation could be analysed the cell was lost. Significant summation effects have been found in the following types of neurones designated by the terms used in Figs. 25 and 27—29: type 1 *B*, type 2 *A*, types 3 *A*, *B* and *C*, type 4 *A*.

As mentioned in the beginning of this section, 8 out of the 27 neurones tested did not display a definite summation, although other signs of an interaction were apparent. As judged from the loud-speaker, the effect in some of these 8 neurones was a breaking up of the discharge rhythm without any significant increase of the response frequency. In one case there were signs of a slowing-down of the discharge frequency in response to maintained muscular stretch. In the remaining cases no certain signs of an interaction could be observed. Summation effects have been more common in some of the cats, a result which might be explained by the poor condition of the spinal cord. It has, however, been possible to observe both definite and less pronounced summation effects in one and the same preparation.

Without attaching undue importance to the fact that it has not been possible to elicit spatial summation effects in all cases, the observation made may, however, indicate that a simultaneous stimulation of two different afferent sources need not necessarily lead to summation. Failure of summation may be due to an unphysiological combination of the receptors activated.

B. *Postsynaptic discharges in response to simultaneous stimulation of an excitatory and an inhibitory source*

In twelve interneurones of convergence types 1 and 2 the interaction of excitatory and inhibitory inflow has been studied in the following way. Standardized stretch was first applied to a muscle group exerting an excitatory effect on the neurone under study. About 20 seconds later constant stretch was applied to a muscle

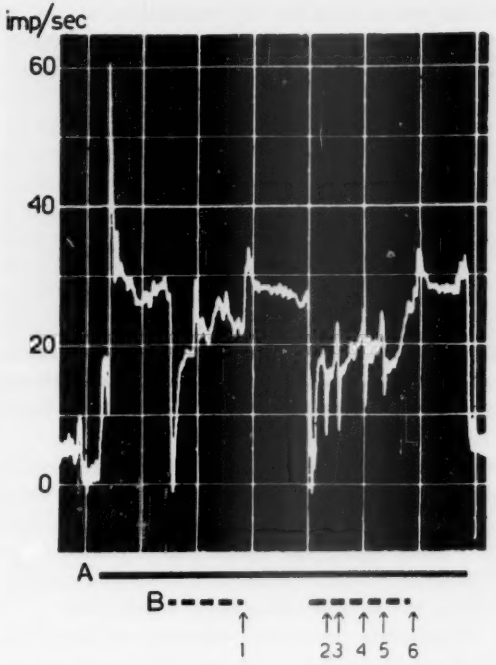

Fig. 35. Reciprocal inhibition. Frequency-time curve obtained from interneurone showing inhibitory action of maintained stretch applied to the cut tendon of quadriceps by load of 300 g (*B*, dotted line) on discharge set up by standardized stretch of biceps-semitendinosus group by maintained maximal extension at knee joint (*A*, solid line). *Arrows: 1*, cessation of inhibitory stimulus with off-effect; *2*, slight blunt pressure of belly of quadriceps muscle; *3—5*, tapping on tendon of quadriceps; *6*, off-effect on relaxation of biceps-semitendinosus. *Time* 30 sec. See text.

group known to inhibit the neurone. This interval between the two stimuli was chosen because after that time the response frequency of the afferent fibres reached a fairly constant level (*cf.* Fig. 8) making it possible to study the effect of the inhibitory inflow without interference from variations in the excitatory inflow. At the same time the average frequency-time curve for the post-synaptic discharge, being the same for excitatory as for inhibitory neurones, reached its slowly "adapting" frequency level.

In these interneurones, the frequency changes induced by the inhibitory stimulus were found to be *the inverted image* of the

frequency-time curve obtained by excitation, *viz.* when an inhibiting muscle is being loaded the response frequency falls rapidly at first but gradually rises again to a fairly constant level.

This is illustrated in Fig. 35. The interneurone could be activated by stretch of the biceps-semitendinosus group (*A*). When a slowly adapting discharge frequency had been reached, stretch of the quadriceps muscle was applied as indicated (*B*) below the figure. The discharge frequency immediately falls to zero but gradually rises again in spite of constant tension of both muscle groups, attaining after about 30 sec a level slightly below the initial frequency level. On relaxation of the muscle exerting the inhibitory action, the response frequency rises to a level corresponding to the adapted frequency before application of the inhibitory stimulus. The response frequency thus adapts to the inhibitory stimulation in a way similar to the corresponding "adaptation" of the excitatory discharge.

As seen in Fig. 35, the response to the constant loading of the biceps-semitendinosus group was being recorded for about 45 sec. The experiment was then repeated with the same load on the quadriceps muscle; again the result was a complete inhibition. When the response frequency started to rise again, a slight pressure was applied to the muscle by a blunt instrument (arrow 2), resulting in a moderate, transient reduction of the discharge frequency. In this way the inhibitory receptors could be traced to a certain muscle group in the same manner as previously described for excitatory receptors (*cf.* part I).

The records in Fig. 35 have been selected as illustrative also of two other effects. It appears from the figure that after cessation of the inhibiting stimulus an *off-effect* is obtained (arrows 1 and 6) in the form of a moderate increase of the response frequency and a subsequent return of the frequency to a constant level. An off-effect ("rebound") of this type could be observed in most neurones tested in this way.

The later, rising phase of the curve (arrows 3, 4 and 5) illustrates the effect of a series of manipulations of the inhibiting muscle performed in order to increase its tension. In each case the effect is a moderate, transient fall of the response frequency, resulting in a prolongation of the total inhibitory period.

By varying the degree of stretch applied to the inhibiting muscle the postsynaptic discharge frequency could thus be modified. Fig. 36 illustrates a closer quantitative study of these variations.

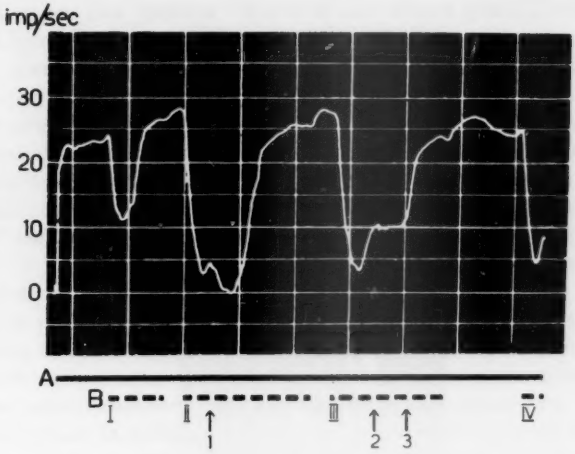

Fig. 36. Graded inhibition, as seen in frequency-time curve obtained from same interneurone and with same experimental arrangement as in Fig. 35. *A*, standardized stretch of biceps-semitendinosus group; *B*, stretch of quadriceps by loading the muscle with different weights, *viz. I* 100 g, *II*, 200 g, at arrow 1 increased to 250 g, *III*, 200 g; between arrows *2* and *3* manual increase of stretch which is then kept constant; *IV*, 200 g. *Solid and dotted lines:* duration of stimulus. *Time* 30 sec. Integrating time constant 5 sec.

The records are from the same neurone but the time constant of the counting rate meter has been larger, so that the rapid frequency fluctuations are not recorded. Also in this case the neurone is being activated by a maintained tension of the biceps-semitendinosus group throughout the experiment (as indicated below the curve). By applying a load of 100 g on the inhibiting muscle, a moderate inhibition is induced, reducing the frequency to 11/sec. (*I*). On relaxation of the muscle so that a constant frequency level is reached again, and on subsequent reloading of the muscle by 200 g, the result is a practically complete inhibition, corresponding to 3/sec. (*II*). When this inhibition begins to adapt (arrow 1), the load is increased to 250 g, which results in a complete inhibition for some seconds, followed again by adaptation. At *III*, the quadriceps muscle has been subjected to a load of 200 g, resulting in a decrease of the discharge frequency to the same level as in *II*. When the adaptation had set in, the response frequency could be kept constant for about 20 sec by manual increase of the tension of the quadriceps (between arrows 2 and 3). The load

on the inhibiting muscle was then kept constant, and the neurone started to adapt again. The inhibiting muscle was then completely released, resulting in a slight rise of the response frequency.

It should be mentioned that a complete inhibition of the response frequency to zero, as shown in Fig. 35, could not be induced in all neurones. In some cases the impulse frequency was only moderately reduced even though the load on the inhibiting muscle group was increased up to 1 000 g. The rates of "adaptation" also differed in different neurones; these observations have, however, not been analysed.

As mentioned in the description of the convergence patterns in part II, a considerable number of neurones excitable by stretch of a muscle group could be inhibited by stimulation of exteroceptive sources. As shown in table II, the exteroceptive sources most commonly observed were pressure receptors in the pads.

The record in Fig. 37 is a typical illustration of how activity induced by muscle stretch may be inhibited by constant pressure applied to the large ipsilateral pad. Maintained stretch of the tibialis anterior muscle (*A*) was first applied. When the response frequency had reached a fairly constant level (in about 45 sec) steady pressure was applied to the pad (*B*), resulting in an immediate fall of the frequency to about 4/sec, followed by a gradual rise up to a level slightly below the pre-inhibition level. A further increase of the pad pressure (arrow 1) again results in inhibition, the frequency declining to about 13/sec. At arrow 2 the pad is completely released, and at arrow 3 the muscle is relaxed. The result is a return of the response frequency to about the same resting discharge as was observed at the beginning of the experiment.

When the pad is released (arrow 2) an off-effect is induced, which is reflected in a sudden increase of the response frequency. The effect is similar to that observed in the experiment shown in Fig. 35.

Constant pressure applied to a pad activates, *i. a.*, slowly adapting receptors (ADRIAN and UMRATH 1929), and the afferent discharge frequency runs a time-course similar to that observed in discharges from muscle receptors of type *A* and *B*, activated by maintained stretch (GRAY and MATTHEWS 1951). As a matter of fact, the inhibitory effect of constant pad pressure resembles the inhibition elicited from a muscular source (*cf.* Fig. 35).

Both in Figs. 35 and 37, the excitatory as well as the inhibitory

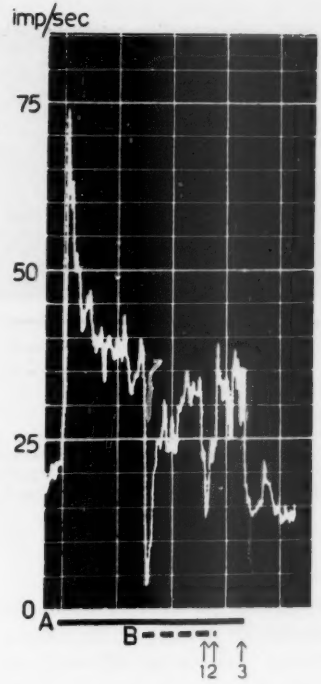

Fig. 37. Frequency-time curve obtained from ventral horn interneurone showing inhibitory action of pad pressure (*B*, dotted line) on discharge set up by standardized stretch of tibialis anterior (*A*, solid line). *Arrows: 1*, pad pressure increased; *2*, pad pressure released (note slight off-effect); *3*, muscle relaxed. *Time* 30 sec.

stimuli have been applied long enough for the afferent impulse frequencies from the various sources to reach constant levels. It is noteworthy that the postsynaptic discharge frequency induced by an interaction of a constant excitatory and inhibitory inflow is only slightly lower than the frequency induced by a purely excitatory adapted inflow.

Before passing on to a description of some other inhibitory features studied, it should be pointed out that the effects described so far (the inhibitory frequency-time curve, the off-effect and the graded inhibition) have been observed in all the twelve neurones in which studies of this type have been performed.

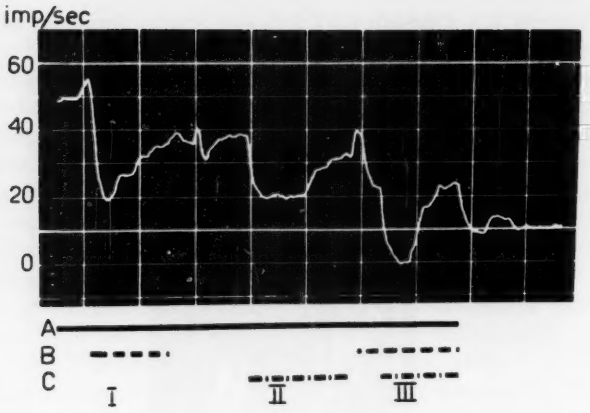

Fig. 38. Inhibitory convergence, as seen in frequency-time curve obtained from single interneurone. *A*, maintained stretch of contralateral triceps surae; *B*, maintained stretch of ipsilateral triceps surae; *C*, pressure applied to ipsilateral pad. *Solid and dotted lines:* duration of stimulus in combinations described. *Time* 30 sec. See text.

Inhibitory convergence

The various convergence patterns found in these investigations provide a rich material for studies of the effects of simultaneous stimulation of afferent sources in different combinations along the lines of the classical reflex physiology. In the analysis of the inhibitory effects, special attention has been paid, *i. a.*, to the features of inhibitory convergence. It is well known from SHERRINGTON's work that a summation of the inhibitory influence from two separate afferent sources may occur, thus increasing the total inhibitory effect. The question then arose whether summation of inhibition could be found to occur in single interneurones.

As only a limited number of neurones with ipsilateral connections were available with a convergence pattern suitable for investigations of this type (see part II), the summation effects have also been studied in neurones with *contralateral* afferent connections. Fig. 38 is an illustrative example of an experiment of this kind. The contralateral triceps surae was subjected to stretch maintained throughout the experiment. The response frequency having reached a constant level, stretch was applied to the ipsilateral triceps surae (I). The inhibitory effect obtained

represents the maximal effect elicitable. II shows the maximal inhibitory effect of pressure on the ipsilateral large pad. In both cases the response frequency is lowered to 20/sec. At III both inhibitory sources have been stimulated simultaneously in the same manner as before; the result is a complete inhibition of the response. It is of interest to observe how even during this strong maintained inhibitory influence the response frequency rises again after some seconds just as described above for other neurones.

DISCUSSION

Before passing on to a discussion of the results obtained in the present work and the conclusions that may be drawn from them, it is essential to consider the limits within which the methods used may be regarded as valid.

Validity of observations. In the first place, the recording arrangements must make it possible to study the activity of the single units for a fairly long period without injuring them. The recording conditions may be considered as satisfactory when the resting potential is stable and the action potentials are of constant size and shape throughout the experiment. A definite sign of serious cell damage is the appearance of high-frequency injury discharges, 600/sec and more; these discharges are further identified by their not being affected by afferent inflow normally influencing the cell. An injury reducing the excitability and thus lowering the normal discharge frequency of the neurone is less easy to reveal but must also be taken into account. One has also to consider that a current flow from adjacent cells injured by the passage of the electrode may have an unphysiological influence on the neurones studied.

As has been pointed out above (p. 14), it is most likely that the group of neurones studied in this work represents a selection based on their tolerance of the intracellular electrode, a tolerance which may to a large extent depend on the size of the neurones. The actual frequency of occurrence of various neurone types cannot be deduced from the observations made in this work, nothing being known about the physiological significance of the cell size. No statistical analysis can be based on the present evidence; it can only be established that neurones displaying these properties exist.

As mentioned above (p. 13), the methods used to distinguish between motoneurones, interneurones and fibres are not fully reliable. Thus, in some cases, neurones included in the material may not have been correctly classified; other neurones may have been excluded on irrelevant grounds. These sources of error have to be taken into consideration in the interpretation of the results.

It should also be kept in mind that the convergence patterns described may not be complete. In some cases, single interneurones with afferent connections with the ipsilateral hindlimb may be activated also from other sources, as for example the contralateral hindlimb, the tail or the forelimbs (KOLMODIN and SKOGLUND 1954 b; FRANK and FUORTES 1956). In regard to most of the neurones described, both the contralateral hindlimb and other parts of the animal, including the forelimbs, have been roughly tested. This being, according to our experience, a fairly reliable way to reveal convergence, most of these neurones are thus, in fact, likely to be influenced only from afferent sources in the ipsilateral hindlimb.

Excessive painful stimulation and supramaximal electrical stimuli seem to be able to open up connections usually closed, thus increasing the number of afferent connections. In the present work, stimuli of this kind have been avoided as far as possible, but a certain inflow from pain receptors due to the fixation arrangements and operative wounds is inevitable.

The experiments have been carried out on spinal cats and all descending cerebrospinal pathways have thus been cut. When passing the spinal cord the electrode may also have cut off some afferent and propriospinal connections of the interneurone under observation, thus disturbing a normal excitatory or inhibitory inflow.

When considering each single observation and its validity, due allowance must be given to the sources of error mentioned above. On the other hand there is reason to assume that the general lines drawn up for the convergence patterns should be at least tolerably correct in view of the fact that a comparatively large number of neurones with similar properties have been found and observed in different experiments. As a further precaution, great care has been taken to exclude all not completely analysed observations, and cell types observed only once have only rarely been included.

Besides these sources of error which are chiefly induced experimentally, variations of the central excitatory state are likely to be of decisive importance for the determination of the convergence patterns. From observations in this work and in other investigations it is evident that the excitability may be extremely varying not only in different neurones but also in one and the same neurone in the course of a recording. In a non-anaesthetized preparation the neurones are hardly ever in a resting condition;

judging from the fluctuations of the membrane potential recorded there is a constant inflow of afferent and propriospinal activity (KOLMODIN and SKOGLUND 1957). For a systematic analysis of the convergence pattern of a single neurone, the recording of these slow potential variations is just as important as the recording of the action potential. In the present work it has not been possible to use such sensitive indices of afferent influence. The convergence patterns determined in this investigation should thus be considered as representing *afferent sources having a dominant influence on the neurone*. This need not necessarily imply that the neurone under observation is activated in a state of facilitation.

There are also other factors in favour of this concept of a dominant influence of certain sources. In some cases, an additional increase of the muscle tension has been seen to reduce the discharge frequency or even to silence the cell, whereas in other cases a relaxation of the muscle has resulted in a sudden increase of the discharge. These observations are difficult to interpret but a possible explanation may be that both inhibitory and excitatory impulses from the same muscle group are fired onto the single interneurones (*cf.* GRANIT and STRÖM 1951), in which case the effect of the stimulation may be dependent on which of these afferent pathways is dominant at a given moment in the neurone under observation.

Convergence. Even from the first studies of convergence in single spinal interneurones (KOLMODIN and SKOGLUND 1954 b; ECCLES, FATT and JANDGREN 1954; 1956) it was clearly evident that interneurones are playing an important role as convergence centres for afferent fibres of the same as well as of different sensory modalities. The analysis of interneurones with proprioceptive connections presented in this work is a more detailed illustration of the significance of convergence for the integrative functions.

It has been possible to establish certain recurrent types of convergence patterns characteristic of the different neurones. Various observations discussed above, especially the fact that there are fluctuations in the excitatory state, suggest that these different convergence patterns do not represent all anatomical afferent connections of the neurone but that they should rather be considered as representing the connections that are passable in the actual experiment, thus implying a functional convergence. It is evident that certain neurones are dominated by one, others

by two and still others by three different muscle groups, whose functional relationship is very close. From a functional point of view it is also interesting to observe the reciprocity apparently prevailing *within* a given type of convergence pattern, for example within the types 2 *B* or 2 *C* (see Fig. 27). It is also evident that the whole system of different convergence patterns seems to be built up on a basis of reciprocity, as seen for instance in Fig. 27 (type 2 *A* contra 2 *B*; type 2 *C* contra 2 *D*) and in Fig. 28 (type 3 *B* contra 3 *C*).

The principle of reciprocal innervation thus seems to be valid not only for motoneurone pools and for single motoneurones but also for the single interneurones influenced by proprioceptive afferent stimulation.

It would seem reasonable to assume that the more complex convergence patterns might be built up out of the simpler patterns, *e. g.* of the types shown in Fig. 25. The only experimental data so far supporting this hypothesis are the recordings from three neurones described on pp. 55–56. The behaviour of one of the neurones described might be explained by the hypothetical scheme illustrated in Fig. 39. The neurone *A* in the figure may be activated by impulses reaching it directly via the pathway M_1. The neurone *A* may also be activated by impulses via the pathway M_2, in which another neurone *B* is intercalated. E_1 is a peripheral source capable of inhibiting the activity in neurone *B* evoked from M_2, which results in abolition of the activity also in neurone *A* (set up from M_2). Stimulation of E_1, on the other hand, does not influence the excitatory action of M_1 on the neurone *A*. According to this interpretation the convergence pattern of the neurone *A* might be supposed to be built up out of the types 1 *A* and 1 *C*.

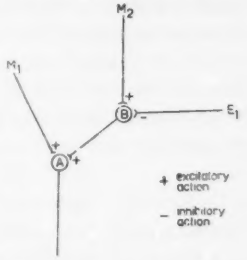

Fig. 39. Illustration of one theoretically possible coupling of interneurones. See text.

The behaviour of the neurone in the experiment described on p. 55 may, however, be explained also in the following way: the different afferent sources M_1, M_2 and E_1 may have direct connection with the neurone *A* via synapses with different location, for instance on the soma or the dendrites. In so far as synaptic trans-

mission is interpreted as being a local process (LORENTE DE NÓ 1938 a), selective interaction may occur within one and the same neurone. The suggestion that the more complex convergence patterns should be built up out of a number of cells of simpler type is thus, for the present, hypothetical.

When classifying the neurones on the basis of the functional organization of the convergence, as in part II above, this classification basis is chosen as being from a functional point of view the most reasonable one out of various possibilities. They can also *e. g.*, be classified into one group in which only excitatory effects have been elicitable and another group in which both excitatory and inhibitory effects could be elicited. This results in two groups of about equal size. It cannot be excluded, however, that the purely "excitatory" neurones may be subject to an inhibitory influence via propriospinal or cerebrospinal pathways. From the present evidence it cannot be decided whether they are fundamentally different from the excitatory-inhibitory neurones, for example as regards their synaptic structure or, if a chemical transmitter is presupposed, as regards its nature. From the point of view of the hindlimb as an afferent functional unit, the existence of excitatory and excitatory-inhibitory neurones is, however, of interest, and implies another aspect of the functional organization.

It has also been established that some of the interneurones studied could be activated from proprioceptive sources only, while others could be influenced both from proprioceptive and exteroceptive sources. These observations suggest still another alternative basis for a classification, *viz.* into two functionally different systems.

The destination of the impulses recorded from neurones in the grey matter is uncertain. They may be mediated to motor nuclei in the same or in adjacent segments, to ascending sensory tracts or to propriospinal systems. That one and the same interneurone may serve different functional systems is evident from CAJAL'S histological pictures (CAJAL 1909) of various types of interneurones, illustrating how a cell may send axon collaterals to various parts of the spinal cord and even to different spinal tracts. Nor is it possible in these investigations, using natural stimulation, to determine whether the connections of the interneurone studied are monosynaptic or multisynaptic. Although

the various convergence patterns found do indeed fit in well with certain reflex systems which are already well defined, *e. g.* the disynaptic linkages studied by LAPORTE and LLOYD (1952) or other polysynaptic reflex systems (*cf. e. g.* LOOFBOURROW and GELLHORN 1949), it is safer not to draw any definite conclusions in this respect. In experiments on motoneurones, KOLMODIN and SKOGLUND (1957) have found convergence patterns of similar types as those observed in interneurones but also in this case the present evidence is not sufficient to permit conclusions as to the functional connections between the interneurones studied and certain types of motoneurones.

There are, however, various possible ways to approach the problem of determining which functional systems the cells may serve; it may be done, for example, by comparative studies of convergence patterns and types of discharges in recordings from cells in the grey matter and ascending tracts. It is of interest that some of the convergence patterns of single neurones in Flechsig's tract described by LAPORTE, LUNDBERG and OSCARSSON (1956) are similar to the patterns described in this paper.

Frequency-time curves. The absolute frequency, the rate of decay of the frequency and the degree of irregularity in the directly recorded frequency-time curves of course reflect the temporal patterning of impulses of the discharges.

Of special interest is the finding that in a given neurone the frequency-time curves established on the basis of responses elicited from the various afferent sources differ considerably; a possible explanation may be that the character of the response is determined by the number of active fibres having connection with the postsynaptic neurone from a certain afferent source, in combination with the impulse frequencies of these fibres. (Other factors may also be of significance which will be discussed later). A detailed analysis of these various discharge types is not within the scope of this investigation. The findings described suggest, however, that the site of the peripheral stimulus may be of considerable importance in determining the character of the postsynaptic discharge frequency.

In this connection it is of interest that studies of the function of larger neurone pools have revealed that the site of the stimulation affects the character of the response. It is known, *e. g.* from reflex studies, that the site of the stimulation affects both the character

and the extent of the movement (SHERRINGTON 1910, *cf.* also HAGBARTH 1952). In their studies of single units in the thalamus, ROSE and MOUNTCASTLE (1954) found that the "modal value" of a repetitive train depended, *e. g.*, upon the locus of application of the stimulus; they suggested that the temporal patterning of the spikes in repetitive trains might play a role in relaying information on the characteristics of the peripheral stimulus.

The existence of *spatial summation*, which is of great interest from various aspects, has previously been studied only in neurone pools but has in the present work been clearly demonstrated in single interneurones.

In part I above, the frequency of the postsynaptic discharges was shown to have a direct relation to the impulse frequency in the afferent fibres within certain limits. The fact that simultaneous stimulation of two different afferent sources results in an increase of the response frequency (spatial summation) may have the very simple explanation that the total number of impulses reaching the neurone has been increased. However, other observations indicate that the explanation is hardly as simple as that. Thus, for example, it has been found that a spatial summation is sometimes reflected in an increase of the initial response frequency without any corresponding increase of the frequency in the latter part of the response. It is also noteworthy that — as appears from Fig. 34 — the characteristics of the summated responses, *viz.* the frequency-time curves of a given neurone, are different depending on which combination has activated the neurone. The response of a given neurone is likely to depend not only on the total number of afferent impulses reaching it but also on the spatial distribution of active synapses on the soma of the postsynaptic neurone or the structural organization of the closed chains involved (LORENTE DE NÓ 1938 a, b).

The different responses obtained in a given neurone during separate stimulation of different afferent sources were supposed to be a possible basis for a mechanism of differentiation of the various sources functionally connected to a neurone. In analogy, it might be suggested that the differences in the summated responses obtained might signal which combination of afferent sources is functioning.

In early electrophysiological investigations on various sense organs (*e. g.* ADRIAN and ZOTTERMAN 1926, MATTHEWS 1933)

the relationship between stimulus strength and discharge frequency in afferent fibres had been established, and in their studies on reflex activation of spinal motoneurones, ADRIAN and BRONK (1929) demonstrated that the frequency of the efferent discharges could also be changed at will by varying the intensity of the natural stimulation. At an early stage it was thus evident from these and from similar findings that *frequency modulation* of nerve impulses constitutes an essential mechanism in central nervous transmission. In the last few years this has also been verified in a number of experiments by direct recording from single units in various parts of the central nervous system. The significance of frequency modulation in spinal interneurones during natural stimulation has been emphasized by KOLMODIN and SKOGLUND (1954 b) and by FRANK and FUORTES (1956).

In the first part of this work it could be demonstrated that in an "average" interneurone there is, within certain limits, a direct proportionality between the postsynaptic discharge frequency and the afferent impulse frequency. However, the postsynaptic discharge frequency was also shown to be dependent on another factor, an "adaptation" process, which may be attributed to the synaptic membrane. In part III it has been established that the discharge frequency during inhibition depends on the intensity of the inhibitory stimulus and, consequently, on the inhibitory afferent impulse frequency, an observation which further supports the concept of a frequency modulation.

However, studies of the discharges in a given neurone during simultaneous stimulation of different converging afferent sources justify the assumption that a third factor is of importance, at least for the frequency of an integrated discharge. In the experiment illustrated in Fig. 34, separate activation of the different sources *A*, *B* and *C* resulted in about the same "adapted" response frequency. The combination *AB* resulted in an increase of this frequency, whereas the combination *BC* did not. Most likely these variations are to be ascribed to differences in the spatial distribution of active synapses on the soma of the postsynaptic neurone or to properties of closed internuncial chains (*cf.* LORENTE DE NÓ 1938 a, b).

The mechanism of frequency modulation in single neurones has not been analysed in this work. The problem has, however, been studied by KOLMODIN and SKOGLUND (1957). In their paper will be demonstrated, that the discharge frequency of a neurone

during natural stimulation could be related to changes of the depolarization level of the neurone.

It has been found that the *inhibitory frequency-time curve* runs a time-course that is the inverted image of the response during excitation. There is also evidence indicating that the inhibitory effect is initiated by impulses from slowly adapting stretch receptors in the muscles. The inhibitory frequency-time curve is, therefore, likely to be dependent on the same factors as determine the course of the excitatory curve.

A long-lasting activity can be set up by maintained activation of an excitatory source. In the same way, maintained stimulation of an inhibitory source results in a long-lasting inhibition; however, the inhibitory response frequency rises in spite of constant inhibitory stimulation, reaching a level only slightly below the pre-inhibition level (*cf.* Fig. 35). The inhibitory effect thus seems to be weaker than the excitatory effect.

A number of the classical concepts in reflex physiology, such as graded inhibition and summation of inhibition as well as of excitation have been demonstrated in single spinal interneurones. These findings provide an experimental proof of SHERRINGTON's concept of the internuncial system as being the site of important integrative processes.

Patterns of activity in an interneurone pool. It has been pointed out that for the solution of the problem of how the spatio-temporal pattern of afferent impulses is transformed in the central nervous system it is essential to have information not only of the properties of the individual neurones but also of their group behaviour (GALAMBOS 1954).

In the present investigation it has been possible to obtain a sampling of different types of interneurones which constitute a functionally uniform group, all neurones being connected with the ipsilateral hindlimb. The behaviour and the convergence patterns of these neurones being fairly well analysed, this group of neurones thus offers an opportunity to illustrate how more complex sensory messages may be transformed in central relay stations.

For this purpose a hypothetical interneurone pool comprising 15 neurones has been constructed, each neurone representing a different type found in the actual experiments. In Fig. 40, the rectangle *E* represents such an interneurone pool and each of the 15 small squares in the rectangle a single interneurone, whose

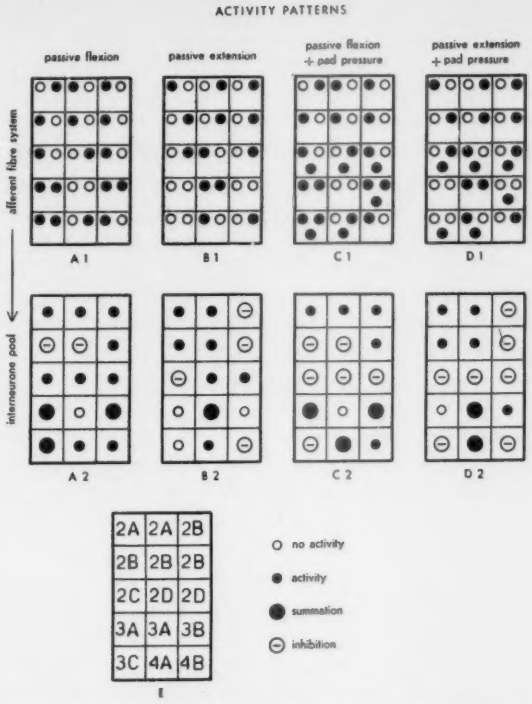

Fig. 40. Activity patterns of hypothetical interneurone pool and the corresponding afferent fibre system under four different stimulus conditions. See text.

convergence type is indicated by the same type-designations (2 *A* — 4 *B*) as were used in part II above. All the neurones have been selected so as to represent different afferent connections; thus for example, one of the two neurones of type 2 *A* was influenced from muscles acting at the ankle joint, the other from muscles acting at the knee joint.

In the rectangles *A* 2 — *D* 2, the small squares represent the same interneurones whose convergence types are indicated in the rectangle *E*. The symbols in these squares illustrate the actual state of activity of each interneurone during various types of afferent inflow (*cf.* below).

The afferent connections of each interneurone are, of course, known, and those engaged in the hindlimb movements marked

at the top of the figure have been indicated by symbols in the corresponding squares in the rectangles *A* 1 — *D* 1. Thus, for example, the interneurone of type 2*A* in the left top square of *E* can be activated from two antagonistic muscle groups. Two symbols have therefore been inserted in the corresponding square of rectangles *A* 1 — *D* 1, indicating the double afferent connection from a flexor and an extensor muscle. Which of the afferent systems is activated depends on whether passive flexion (*e. g. A* 1) or extension (*e. g. B* 1) is performed (filled circles marking activation). Another example: if an interneurone receives inflow from two synergistic extensor muscles (type 3 *C*, left bottom square in *E*) two afferent pathways are activated by one type of movement (the two filled circles in *A* 1 left bottom), whereas the opposite movement leaves both fibre systems unaffected (the open circles in *B* 1 left bottom).

The rectangle *A* 1, as a whole, thus gives a survey of the activity in the afferent connections of all the 15 interneurones during a passive flexion of the ipsilateral hindlimb at both knee and ankle joints. During passive extension, on the other hand, the activity pattern obtained is the inverse (*B* 1), the previously active afferent fibre systems being inactive and *vice versa*.

As far as the afferent patterns of activity are concerned, there are only two alternatives — activation or no activation —, whereas for interneurones the pattern of activity is more variable, as shown in the rectangles *A* 2 and *B* 2. In some of the interneurones also summation and inhibition effects appear, depending on the functional organization in each case. A comparison of the patterns of activity of the whole neurone pool shows that a change from one type of leg movement to the other gives rise to characteristic asymmetrical patterns.

These were examples of simple combinations of afferent activation, consisting of a proprioceptive inflow only. If, however, to the same passive limb movements is added a simultaneous pressure of the pad, activity is set up, as far as some of the interneurones are concerned, also in another afferent system, *viz.* from exteroceptors of the pad. This is illustrated in the rectangles *C* 1 and *D* 1, where a third filled circle has been included in the squares representing these neurones. As a consequence of the different afferent inflow there is a definite characteristic change also in the pattern of activity of the interneurone pool (*C* 2 and *D* 2).

It should be pointed out that in these schemata the pattern

of activity of each individual interneurone is based on experimental observations in different preparations and only the combination of the types chosen into an interneurone pool is hypothetical. If one assumes that interneurone pools of similar composition actually exist as functional units in motor or sensory systems, these schemata may give a concept of the patterns of activity effective in such systems.

Even from these simple schemata it is evident that in the interneurone pool, as a whole, there may appear patterns of activity that are unique for certain movements or positions of the limb. It is obvious that even a small number of postsynaptic neurones — as compared with the total number of afferent fibres — have many possibilities to transmit complex messages to subsequent destinations.

SUMMARY

1. The main object of the present work has been to study the function of single spinal interneurones with proprioceptive connections by means of natural stimulation in non-anaesthetized preparations of decapitate cats; the results are based on recordings from about 200 interneurones that could be influenced by passive stretch of de-efferented muscles in the ipsilateral hindlimb. As standard stimulus has been chosen stretch of a muscle or a muscle group corresponding to maximal flexion or extension at the corresponding joint (*"standardized stretch"*). Exteroceptice inflow has been produced by application of touch, pressure and pain stimulation. Action potentials from single interneurones and afferent fibres have been obtained by intracellular recording, using glass capillary microelectrodes. By means of a counting rate meter and an inkwriter the discharge frequency has been recorded directly in the form of frequency-time curves. The position of the recording points in the spinal cord has been determined by histological methods. The criteria for identification of various types of spinal neurones and the range of validity of the methods used are discussed.

2. In order to study the *afferent inflow* during standardized stretch the discharges in single muscle afferents have been recorded and the results compared with data known from earlier investigations. Typical frequency-time curves of discharges from slowly adapting muscle receptors have been described, as well as variations observed in such curves from different fibres in one muscle or in different muscles. The average afferent frequency-time curve in response to standardized stretch has been calculated on the basis of recordings from 75 different fibres of slowly adapting stretch receptors from three different muscle groups (Fig. 8). The mean value of the turning point of the curve — which is a rough measure of the rate of decline of adaptation in the receptors — was found to be 13.5 ± 0.8 sec.

3. Two main types of *interneurone responses* were observed during maintained stretch of a muscle or muscle group, *viz.* sustained and transient discharges.

Sustained discharges were observed in 90 % of the interneurones studied. In about half of these interneurones studied a resting discharge was observed which in 50 % of the cases could be traced to peripheral activity. The sustained discharge has a comparatively high initial frequency, declining discontinuously to a lower, comparatively constant or only slowly falling frequency level. In different neurones great variations were found in the course of the curve and in its degree of irregularity. The effect of variations in the rate and magnitude of stretch has been studied, as also the effect of rapid release of stretch. The receptors were localized by blunt pressure or by dissection; in 60 % of the cases they were found in the belly of the muscle. The conclusion is drawn that this type of sustained discharges is set up by slowly adapting stretch receptors in the muscle (both muscle spindles and tendon organs).

The average interneurone frequency-time curve (Fig. 20) has been calculated on a material consisting of 67 interneurones which have been selected from the same experiments in which the afferent inflow was studied. The mean value of the turning point of the curve was found to be 24 ± 0.9 sec.

Transient discharges consisting of a burst of impulses usually ceasing within 1—5 sec were observed in 10 % of the interneurones studied. In some cases this type of response could be traced to receptors in the fascia of the muscle.

4. On the basis of the average frequency-time curves a *comparison has been made between sustained afferent and postsynaptic discharges.* It was found that during the first 12 sec after onset of the discharge the relation between the average afferent and the average internuncial impulse frequencies was, roughly, one of direct proportionality. When the average afferent curve had reached a fairly constant level there was still a continued frequency fall in the interneurone curve ("adaptation of the average interneurone"). A statistical analysis showed that the differences in the two curves in this respect are highly significant. The interneurone discharges were always irregular, in contrast to the regular afferent discharges.

5. The *patterns of convergence* of 89 individual interneurones have been determined by a systematic mapping of the various afferent sources in the ipsilateral hindlimb from which the neurones could be influenced. The different convergence patterns found so far are of five main types (Figs. 25, 27—29): some neurones

could be influenced from one muscle group only (type 1), others from two different muscle groups acting as antagonists at the same joint (type 2), as synergists at neighbouring joints (type 3) or as antagonists at neighbouring joints (type 4). A small group could be influenced from three different muscle groups (type 5). More than half of the neurones could also be influenced from different exteroceptive sources. The histological localization of the interneurones of various types has been indicated in diagrams of spinal cord cross sections.

6. Among the neurones with the same type of convergence pattern, cells of different functional properties could be distinguished. Thus, in one group separate activation of the afferent sources evoked *only excitatory effects*. In a given neurone of this type the time-course of the response elicited from one afferent source or combination of sources might differ considerably from the response evoked by stimulation of another afferent source or combination of sources.

7. In 19 out of 27 neurones tested, simultaneous stimulation of two excitatory afferent sources resulted in a higher response frequency than separate stimulation of each of the sources. Usually there was an increase of frequency both in the initial and the later phase of the response, whereas in some cases an increase was observed either in the initial or in the later phase. In 8 of the neurones tested no definite summation was found.

8. In another group of cells *both excitatory and inhibitory effects* could be observed. The discharges set up from one afferent source could be inhibited — completely or partially — by simultaneous stimulation of another peripheral source which, when stimulated alone, did not elicit a postsynaptic response. During maintained inhibitory stimulation — studied in 12 neurones — the response frequency was found to fall rapidly at first but gradually rose again to or slightly below the pre-inhibition level. As a general principle it was found that this frequency-time curve was the inverted image of an excitatory curve. The frequency of the response might be modulated by varying the intensity of the inhibitory stimulus. A summation of the inhibitory influence from two afferent sources has been found to occur in single interneurones, thus increasing the total postsynaptic inhibitory effect.

9. In the discussion of the functional significance of the results special attention has been paid to the patterns of convergence

found and to the behaviour of individual interneurones expressed in frequency variations. Interneurones of different types actually observed were combined into a *hypothetical neurone pool* and various activity patterns were deduced and schematically represented to illustrate a possible mechanism for central transmission of complex sensory messages.

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INTEGRATIVE PROCESSES IN SINGLE SPINAL INTERNEURONES WITH PROPRIOCEPTIVE CONNECTIONS

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